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Growth Through Agricultural Progress

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Paul R. Miller

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CONTENTS

USE OF A PORTABLE INOCULATION TOWER IN LABORATORY, GREENHOUSE, AND FIELD TESTS OF FUNGICIDES TO CONTROL RICE BLAST

M. M. Kulik and G. N. Asai

Abstract

A portable inoculation tower was used to test a number of fungicides in the laboratory, greenhouse, and field for control of rice blast. It was used in the laboratory tests to disperse spores of Piricularia oryzae in Petri dishes that contained the fungicides spread on the surface of water agar. In the greenhouse and field tests, the tower was used to inoculate rice plants previously sprayed with fungicides. Eight of 24 fungicides screened in the laboratory were further tested in the greenhouse; 5 of these were later evaluated in the field. Of these 5 materials, 3 gave promising control of rice blast.

INTRODUCTION

Tests were undertaken of some of the newer fungicides[1] to find a possible control for rice blast. In carrying out these tests, it was necessary to inoculate rice plants with spores of the fungus pathogen, Piricularia oryzae. Inoculations were made using dry spores disseminated inside an inoculation tower placed over the test plants. This method insured a relatively even distribution of spores and confined the inoculum to the desired plants. Because of the tower's design and light weight it was easily used in flooded rice fields as well as in the greenhouse.

DESCRIPTION OF THE PORTABLE INOCULATION TOWER

The portable inoculation tower used was a simplified, portable version of the settling tower developed at this laboratory (1, 2, 3). It consisted of four rectangular sides plus a roof and was constructed of 1/4-inch finished plywood. It was 36 inches wide, 40 inches deep, and 57 inches high, and could be assembled or broken down into its component parts in less than 30 minutes. The tower was fitted on one side with two small ports, one through which to insert the muzzle of a carbon dioxide pistol and the other for observation. It was also equipped with a pair of wooden handles so it could be easily moved about in the greenhouse and field. The entire structure was given three coats of Waterlox inside and out, and an additional outside coat of aluminum paint.

The inoculum was disseminated inside the tower with a Crosman carbon dioxide pistol fitted with an aluminum barrel extension; this extension was bent up at a right angle 4 inches back from the muzzle opening and was designed to fit into a slot cut in an aluminum brace attached on the inside of the tower. In use, the inoculum was simply poured into the muzzle, which was then fitted into the brace inside the tower and thus held firmly in place during the firing of the pistol.

Preliminary tests conducted in the greenhouse showed that a good distribution of spores was obtained inside the tower and that 20 minutes was sufficient time for the spores to settle out of the air.

LABORATORY SCREENING

Procedure: Small Petri dishes containing 1% water agar were filled with solutions or suspensions of 24 of the newer fungicides. The dishes were then rotated and emptied, thus leaving a film of the fungicide solution or suspension on the agar surface. Each fungicide was used to treat four dishes. The dishes were next placed in the portable inoculation tower and spores of Piricularia oryzae were disseminated in the tower at a rate sufficient to allow ease of counting under the microscope. Finally, the dishes were collected and placed in an incubator at room temperature for 4 hours, after which they were examined with the high-power objective of the microscope; 30 fields per dish were examined at random and the number of germinated and nongerminated spores was recorded. The criterion for germination was the presence of a germ tube at least as long as the shortest diameter of the spore.

Results: The results of the laboratory screening tests are presented in Table 1.

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[1] Kindly furnished by the following: American Cyanamid Company, California Spray-Chemical Corporation, Chemagro Corporation, Chipman Chemical Company, Inc., Mallinckrodt Chemical Works, Metalsalts Corporation, Naugatuck Chemical, Olin Mathieson Chemical Corporation, Panogen Company, Rohm & Haas Company, Stauffer Chemical Company, and Union Carbide Chemicals Company.

Table 1. Percentage of spores of Piricularia oryzae that germinated in water agar tests of a number of fungicides.

Fungicide[a]	Concentration (in ppm) 1000	200	50	10	0
Test 1					
Maneb (70%)	0	0	0	0	
Kromad	0	0	0	0	
Dyrene	0.7	0	0	0	
Ortho Phaltan 50W	0	0	0	0.8	
Thioneb 50W	0	0	0	3.3	
Panogen Soil Drench	0	0	0	12.8	
Chemagro D-113	0	0	0	17.8	
Panogen Turf Spray	0	0	12.3	20.0	
Merbam 10	0	0	11.0	22.3	
Cadminate	0	8.0	8.3	24.8	
Dodine (65%)	2.0	23.0	12.0	26.0	
Amer. Cyan. E.F. 23441	16.0	23.0	15.8	27.8	
Check (water)					36.8
Test 2					
Meta-Sol M	0	0	0	0	
Omadine, ferric salt	0	0	0	0	
Phenyl Mercuric Acetate	0	0	0	1.0	
Omadine, disulfide salt	0	0	0	1.0	
Captan (50%)	0	0	0	2.8	
Calo-Clor	0	0	0	11.0	
Chipcote 25	0	0	0	11.0	
Maneb (70%)	0	0	0	12.0	
Chemagro B-1843	0	0	9.6	12.3	
Phenyl-Hg-Propionate	0	0	0	14.0	
Phenyl-Hg-Oxyquino-linate	0	0	0	15.5	
Olin Mathieson Exp. Fung. 1763	0	0	26.1	11.0	
Check (water)					31.0

[a] Chemical formulations for proprietary fungicides as follows: Kromad= cadmium sebacate 5%, potassium chromate 5%, malachite green 1%, auramine 0.5%, thiram 16%; Dyrene= 2,4-dichloro-6-(o-chloroanilino)-s-triazine; Ortho Phaltan 50W= N-trichloromethyl thiophthalimide; Thioneb 50W= polyethylene thiuram sulfide; Panogen Soil Drench= methylmercury hydroxide 3.5%; Chemagro D-113= 1,2-dichloro-1-(methylsulfonyl) ethylene; Panogen Turf Spray= methylmercury dicyandiamide 2.2%; Merbam 10= phenyl mercury dimethyl dithiocarbamate 10%; Cadminate= cadmium succinate 60%, 29% cadmium; EF-23441= N-dodecylguanidine acid phthalate 70% WP; Meta-Sol M= methylmercury 8-hydroxyquinolinate; Omadine, ferric salt= 2-pyridinethione 1-oxide; Calo-Clor= mercurous chloride 60%, mercuric chloride 30%; Chipcote 25= methylmercury nitrile 5.41%; Chemagro B-1843= trans-1,2-bis(n-propylsulfonyl) ethylene, 50%WP; Olin Mathieson Exp. Fung. 1763= chloroacetaldehyde-2,4-dinitrophenylhydrazone.

GREENHOUSE SCREENING

Procedure: Twelve pots, each containing five to seven plants of rice variety CI 8970, were used to test 8 of the 24 fungicides previously evaluated in the laboratory. The plants were sprayed with the fungicide solutions or suspensions and were allowed to dry in place on the floor of the spray room. The check plants were sprayed with tap water. All plants were then taken into the greenhouse and inoculated with spores of P. oryzae at the rate of 2 milligrams of pure, viable spores per square yard, by means of the portable inoculation tower. After inoculation, the plants were placed in dew chambers (4) for 16 hours at 80° F. They were then removed and placed in the greenhouse for 6 days to await the development of blast lesions.

Results: Eight fungicides were tested at concentrations of 1000, 200, and 50 ppm. The results of these tests are presented in Table 2.

Table 2. Average number of blast lesions observed on rice plants sprayed with different fungicides in greenhouse tests.

Fungicide	Fungicide sprayed at: 1000 ppm	200 ppm	50 ppm	0 ppm
Phenyl Mercuric Acetate	*[a]	0.0	0.0	
Ortho Phaltan 50W	0.02	0.35	3.48	
Omadine, disulfide salt	0.16	0.25	4.2	
Meta-Sol M	0.0	0.0	20.1	
Dyrene	0.0	0.13	25.28	
Maneb (70%)	0.01	0.0	41.11	
Omadine, ferric salt	19.3	13.7	21.6	
Kromad	6.4	13.3	54.72	
Check (water)				40.6

[a] Caused extreme phytotoxicity, no lesions observed.

FIELD SCREENING

Procedure: Five of the eight fungicides evaluated in the greenhouse at Fort Detrick were tested in the field at Beaumont, Texas. The experimental design used in several of the rice paddies of the Texas Rice-Pasture Experiment Station was a modified randomized block design, with each treatment replicated four times. Rice plants of variety CI 8970 were sprayed with the fungicides with a hand-operated Hudson tank sprayer. The fungicides were tested at 200 ppm and were sprayed on the plants until droplets just began to run off. One tenth ml of wetting agent, Triton B-1956, was added to each liter of solution or suspension.

The plants were inoculated late in the day after the fungicide spray had dried. The portable inoculation tower was employed in the field to inoculate the sprayed plants with spores of *P. oryzae* at the rate of 2 mg of pure, viable spores per square yard.

It was hoped that these tests could be put out on successive days in order to increase the probability of securing infection on at least 2 or three days. However, inclement weather prevented this and instead, tests were carried out on 7 non-consecutive days. Unfortunately, infection was secured on only one of these days.

Results: Three of the five fungicides kept the amount of infection down to an average of less than one lesion per plant (Table 3).

Table 3. Average number of blast lesions observed on rice plants sprayed with fungicides in the field.

Fungicide (200 ppm)	Total number of lesions	Total number of plants	Average number of lesions per plant
Phenyl Mercuric Acetate	11	96	0.11
Ortho Phaltan 50W	42	126	0.33
Dyrene	54	105	0.51
Meta-Sol M	130	131	1.00
Omadine, disulfide salt	271	115	2.36
Check (water)	354	115	3.08

DISCUSSION

The portable inoculation tower described in this paper is inexpensive, simple in construction and operation, yet well suited to inoculation of rice in the laboratory, greenhouse and field. It is easily manipulated in the flooded rice paddy where only light equipment can be taken. Most important of all, the tower affords uniform inoculation of the rice plants in the plot enclosed by the tower and between plots inoculated.

Phenyl Mercuric Acetate appears to be the most effective compound in controlling rice blast. However, because of its phytotoxicity and the possibility of toxic residue in the grain, other fungicides tested might eventually prove more desirable.

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U. S. ARMY CHEMICAL CORPS, FORT DETRICK, FREDERICK, MARYLAND

SCAB IS NOW AFFECTING THE STUART VARIETY OF PECAN IN GEORGIA AS WELL AS IN OTHER SOUTHEASTERN STATES

John R. Cole[1]

Economically, the Stuart may be called the backbone of the propagated, or improved, varieties of pecan grown in Louisiana eastward to the Atlantic Coast. Therefore, in 1955 when Cole and Gossard[2] first found scab (Fusicladium effusum Wint.) affecting the nuts of the Stuart near Laurel and Lumberton, Mississippi, it was cause for concern by research workers as well as by pecan growers and processors. Either the strain of the fungus causing scab was new or the Stuart variety had suddenly become highly susceptible, because by 1956 scab had spread to several additional orchards in western Louisiana and Mississippi, as reported by Cole and Gossard[3]. Spread of scab has continued and in 1961, in addition to causing serious losses to the Stuart variety in many areas of Alabama, scab is now affecting Stuart nuts in southwest Georgia (Fig. 1). This finding indicates rapid spread of the fungus, since in 1956 the writer and Gossard had not found scab on Stuart nuts east of Fruitland Park, Mississippi[3]. Therefore, a vigorous spray program seemingly should be inaugurated to control scab on the Stuart, as well as on the Schley and other highly susceptible varieties of pecan.

FIGURE 1. Cluster of Stuart pecan nuts affected by scab, collected in orchard near Baconton, Georgia. Nuts on Stuart trees had remained free of scab in this locality until the growing season of 1961.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, ALBANY, GEORGIA

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[1]Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Albany, Georgia.

[2]Cole, John R., and A. C. Gossard. 1956. Stuart pecan found to be susceptible to scab in Mississippi. Plant Disease Reptr. 40: 156.

[3]Cole, John R., and A. C. Gossard. 1956. Increased virulence of scab (Cladosporium effusum Wint.) Demaree) on Stuart pecan in Mississippi and its presence in Louisiana. Plant Disease Reptr. 40: 1120.

THE PROBABLE COIDENTITY OF THE MORIA DISEASE OF PEAR TREES IN ITALY AND PEAR DECLINE IN NORTH AMERICA[1]

Thomas A. Shalla, Luigi Chiarappa, E. C. Blodgett, Elvio Refatti, and Elio Baldacci[2]

Summary

A study was made to determine if the disease called "moria" (plague) which killed over 63,000 pear trees in the Trentino-Alto Adige district of Italy between 1944 and 1947 was the same as pear decline, currently a destructive disease of pear trees in western North America. Orchards were visited in the Trentino-Alto Adige and Ferrara-Mantova pear-growing districts of Italy. Symptoms of diseased trees were observed and samples of bark were prepared for microscopic determination of phloem necrosis. Trees presently affected with moria exhibited foliar symptoms identical to those of the slow form of pear decline. Other similarities to pear decline included reddening of foliage in the summer, brown discoloration and degeneration of phloem tissue below the graft union, lack of feeder roots, and depletion of starch in larger roots. The manner of disease development in Italy was found to parallel closely the development of pear decline in North America. It was concluded that, at present, there is no reason to believe moria and pear decline are not the same disease. However, the coidentity must be viewed as presumptive until the causal factors are known.

INTRODUCTION

Pear decline is currently the most destructive disease of pear trees in western North America. It is estimated that over 100,000 trees were killed by this disease in California alone (9) during the 1960 growing season. Trees die by a gradual loss of vigor over a period of several years (slow decline), or by a sudden wilting in a matter of days (quick decline). The cause of the disease is not known.

Pear decline was first reported in British Columbia in 1948 (8) and subsequently appeared in Washington, Oregon, and California (9). A comprehensive report on studies of a similar condition called "moria" occurring in northern Italy was published in 1949 by Baldacci, et al. (1). Approximately 63,000 trees were killed by this disease in Italy between the years 1944 and 1947. Symptoms resembled those of pear decline but from the literature alone it was not clear whether the two diseases were the same.

In the summer of 1961, through an exchange of visits, the authors were able to observe and compare the two diseases both in North America and the major pear-growing districts of Italy. The latter are located in the valleys of the Adige River and its tributaries (Trentino-Alto Adige) and in the provinces of Ferrara and Mantova in the lower Po River Valley (Fig. 1). This report describes the results of these observations and reviews previous studies relating to the nature and cause of the diseases.

SYMPTOMATOLOGY

Symptoms of moria on the varieties Williams' Bon Chrëtien (Bartlett), Kaiser Alexander (Bosc), Moscatella piccola d'estata, and Butirre Giffard were as follows:

Foliage: Diseased trees varied in appearance. Some had only slightly limited growth and small pale leaves, while others had no terminal growth and very sparse foliage (Fig. 2). Trees affected with moria appeared identical to those in North America affected by the slow phase of pear decline (Fig. 3). Although some trees suddenly wilted and died during 1944-47, no symptoms of quick decline were found during the present survey. All of the disease was of the slow decline type.

Most diseased trees were uniformly affected, exhibiting no "one-sided" effect. One Bartlett tree that was more severely affected on one side than the other was found to have a large scion root on the side of the tree that was more vigorous.

The foliage of trees affected by moria turns abnormally red in the summer. Several trees were observed near Trento during the last week of June that had symptoms of moria and had bright red foliage. Abnormal reddening of pear foliage has been associated with pear decline (4).

[1]This study was supported in part by funds furnished by Pear Zone No. 1, the California marketing program for quality control, merchandising, and research on canned Bartlett pears, and Public Law 480 funds provided by the United States Department of Agriculture.

[2]Respectively, Assistant Plant Pathologist, University of California, Davis; Plant Pathologist, Di Giorgio Fruit Corporation; Plant Pathologist, Washington State University and Washington State Department of Agriculture; Plant Pathologist, University of Milan; Professor of Plant Pathology and Director of the Instituto di Patologia Vegetale, University of Milan.

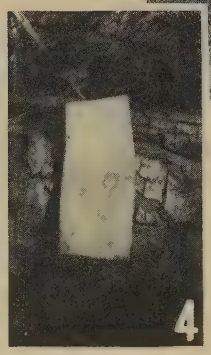

FIGURE 1. Map of northern Italy showing distribution of moria in 1945-47 and as observed in 1961.

FIGURE 2. An approximately 20-year-old Bartlett pear tree near Trento, Italy with typical symptoms of moria.

FIGURE 3. Bartlett pear tree near Sacramento, California with symptoms of pear decline.

FIGURE 4. Brown line at the graft union on the cambial face of a bark piece removed from a Bartlett tree with moria disease near Trento.

FIGURE 5. Six- to eight-year-old Bartlett pear tree with symptoms of moria in Trentino-Alto Adige.

Roots: The root systems of several trees with moria were examined. Most of the feeder roots were dead, while the larger roots were alive and apparently in good condition. No other source of root trouble was found. Trees with pear decline are similarly affected.

Graft Union Phloem: The most reliable diagnostic symptom of pear decline is a characteristic degeneration of the phloem immediately below the graft union (2, 11). This degeneration may be detectable only by microscopic examination of stained bark sections, or it may be visible as a dark brown line on the cambial face of the graft union phloem. Strips of bark were removed from the vicinity of the graft union on 45 diseased and 23 apparently healthy pear trees in the two growing districts of Italy. Each sample was observed for the presence of a brown line and then killed, sectioned, and stained for microscopic study following the method described by Schneider (12).

Thirty-one of the 45 diseased trees had degenerate phloem at the graft union characterized by sieve tube necrosis, ray distortion, and formation of abnormal replacement phloem. There was no apparent difference between the condition of the phloem of trees affected with moria and the phloem of trees with pear decline. Fifteen of the diseased trees with degenerate phloem had a distinct brown line at the graft union (Fig. 4). Twenty-one of 23 apparently healthy trees had normal phloem at the graft union.

Phloem necrosis was found in trees growing in both Trentino-Alto Adige and Ferrara-Mantova districts.

Starch: Baldacci, et al. (1) reported that roots of trees with moria gave a negative starch reaction while there was an accumulation of starch above the graft union. They suggested that this may be the result of phloem necrosis. Similarly, Batjer and Schneider (2) reported that trees affected by pear decline in Washington consistently had an accumulation of starch above the graft union and a depletion below. Critical starch determinations in trees affected with moria were not made in the present study.

EPIDEMIOLOGY

A report by Mader (7), and observations by Catoni (6), suggest that moria was present in Trentino-Alto Adige as early as 1908. However, it did not reach a serious level until 1944-47. The percentage of trees affected in the area during the epidemic varied widely among orchards. Baldacci, et al. (1) reported typical figures of 3, 14, and 28 to 35% in the first, second and third years, respectively. There was no definite pattern of spread in any of the orchards studied. Following the severe epidemic, only a few scattered cases of moria were found. Although moria was reported to occur only in Trentino-Alto Adige (1), in the present study the disease was also observed in the Province of Mantova (Fig. 1).

During the years the disease was most severe, moria was most frequently found in trees 10 to 40 years of age and was not found on nonbearing young trees. Similarly, in the present survey, most of the trees affected were 10 to 40 years old. One 6- to 8-year old Bartlett tree had typical symptoms of moria, including brown line at the graft union (Fig. 5).

Tree variety did not appear to be related to the occurrence of moria, although some early producing varieties such as Moscatella piccola d'estata and Bartlett may be more susceptible to the disease (1). The Bartlett variety was most widely cultivated in the Trentino-Alto Adige district and was the most common variety affected.

The relationship of moria to rootstocks is not clear. Studies in North America (2, 3, 5) indicate that trees on Oriental rootstocks (Pyrus serotina and P. ussuriensis) are most susceptible to pear decline. However, there is no record of commercial use of these Oriental rootstocks in Italy. Studies by Baldacci, et al. (1) and observations made during the present study indicate that many of the affected trees in Italy were on French rootstocks (presumably P. communis). Exact sources of seed used for rootstocks of pear trees in Italy are not known and need further study.

Trees currently affected by moria take several years to die (1). Several severely affected trees observed near Trento were reportedly healthy the previous year.

ETIOLOGY

Extensive studies by Refatti (10) and Baldacci, et al. (1) failed to reveal the cause of moria. No fungus, bacterium, or insect was found associated with the disease. No relationship was demonstrated between the disease and war chemicals such as phosphorous and chlorohydrin, or pesticides containing lead, arsenic, or sulfur. Climatic conditions, cultural practices, water relations and nutrition appeared to have no causal relation to the disease. Diseased trees did not respond to injections of growth regulating materials or 8-hydroxyquinoline sulfate. Results of experiments aimed at transmitting a possible causal virus, either by grafting or by juice inoculations, were negative after 2 years' observation. Despite these negative results, the auth-

ors concluded that moria possibly may be due to a virus because of the exclusion of other possible causes and because of the similarity of moria to virus diseases of fruit trees such as citrus quick decline and cherry buckskin. Up to this present survey, no research work has been conducted on this disease in Italy since 1949.

CONCLUSIONS

The following similarities were found between the moria disease of pear trees in Italy and pear decline in North America, either by direct observation or from the literature.

Symptoms: a) Slow and quick decline foliar syndromes; b) normal appearing main root structure with lack of feeder roots; c) abnormal red foliage in the summer; d) brown line and phloem necrosis in the graft union bark; e) depletion of starch in roots and accumulation of starch above the graft union.

Epidemiology: a) Sudden outbreak followed by decreased incidence of disease. This is similar to the pattern of pear decline in the State of Washington in recent years. b) Some quick decline at the onset of the disease followed predominantly by slow decline in later years. Although there was a high incidence of quick decline in Washington during the early years of disease development, in recent years there has been little (13). c) Tree age (primarily trees of the 10- to 40-year age group are affected). d) Little or no difference in susceptibility among varieties.

There are no apparent differences between moria in Italy and pear decline in North America. Conclusive proof that the two diseases are the same will lie in a demonstration that both are caused by the same factor or combination of factors. At the present time, however, there is no reason to believe that they are not the same.

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UNIVERSITY OF CALIFORNIA, DAVIS; DI GIORGIO FRUIT CORPORATION; WASHINGTON STATE UNIVERSITY AND DEPARTMENT OF AGRICULTURE; UNIVERSITY OF MILAN; AND INSTITUTO DI PATOLOGIA VEGETALE, MILAN

A GENERALIZED LIFE CYCLE OF PATHOGENS OF TREES[1]

Charles L. Fergus and William J. Stambaugh[2]

Although there are two excellent textbooks on forest pathology available in the United States (1, 2), neither contains a satisfactory generalized life cycle of pathogens of trees. Boyce (2) gives enough information about causal agents of specific diseases to allow one to be constructed. However, a developmental life cycle illustrating principles which may be applied to the life cycles of either dwarf mistletoe, leaf spot, wilt, or canker pathogens, is of considerable aid to the forestry student in obtaining a broad perspective of forest pathology.

A generalized life cycle has been developed by the authors (3) over a number of years. It is presented here with the hope that it may aid both the teacher and the student of tree diseases. Comments and suggestions for its improvement will be welcomed.

A life cycle is the series of changes through which an organism passes in the course of its development. It is important to know life cycles of pathogens because the weakest link in the chain of events may be determined and broken, thus giving the most effective control.

The first phase of life cycles of pathogens to consider is the method of overwintering of the pathogen. Most pathogens pass the winter, in Pennsylvania at least, in a dormant resting stage adapted to withstand the adverse environmental conditions. Some of the means by which fungus pathogens overwinter are: fruiting bodies, such as perithecia, pycnidia, and apothecia; highly resistant spores, such as oospores, chlamydospores, and teliospores; and vegetative structures, such as sclerotia, rhizomorphs, and mycelium in perennial parts of the tree. With the return of weather conditions conducive to plant growth, the hibernating pathogen may renew its vegetative development or may immediately sporulate, releasing inoculum which is easily separated and spread to start new individuals.

The place in which a pathogen overwinters is a characteristic of the specific pathogen and its suscept. The usual locations for tree pathogens are: in dead fallen leaves and twigs, in the soil, in perennial parts of trees, such as roots, bark, and twigs. Thus, infected trees, plant debris on the forest floor or in the soil, and infected alternate hosts are sources of inoculum in the spring to start primary life cycles (cycles started from inoculum produced after a dormant period, in contrast to secondary cycles which are started from inoculum produced during the current growing season).

The inoculum is formed at its source under certain required environmental conditions of light, temperature, and moisture. After formation, the inoculum (usually spores or seeds) must be moved in some way from the place of formation to the specific part of the tree through which it normally gains entrance to initiate the disease. This point of entry may be called the infection court. The indiscriminate movement of inoculum is dissemination. Some of the methods of dissemination are:

a. Wind, rain splash, insects, pruning implements, birds, rodents.
b. Infected stock transported, and planted by man.
c. Pathogen spread by its own activities, such as: spores swimming in soil water, mycelium or rhizomorphs growing through the soil from diseased to healthy trees, and seeds forcibly discharged.
d. Spores pulled through, or mycelium growing through, root grafts.

After the inoculum arrives at the suscept, it must grow or produce a thallus, and in some manner gain entrance into the tree. If spores or seeds are the inoculum, they must germinate. Environmental factors are critical at this point in the life cycle, for most spores require precipitated moisture for germination. Temperature, light, food and toxic substances on the tree surface, and other factors, are important. Entrance into the tree may occur in several ways: by direct penetration through intact plant surfaces, through natural plant openings, through root grafts, and through wounds. The latter is the most common mode of entry.

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[1]Contribution No. 240 of the Department of Botany and Plant Pathology, Pennsylvania State University, University Park, Pennsylvania.

[2]Associate Professor of Botany and Plant Pathology, and Assistant Professor of Forest Pathology, respectively.

Once the pathogen enters, colonization or invasion of the tissues of the plant follows. As a result of the presence of the pathogen, physiological interaction results. The first evidence of infection is basically the observed response of the invaded plant to the physiological disturbance, which is seen as a symptom or symptoms.

Most pathogens associate themselves with the suscept to obtain nutritive substances and water. They may be internal, with intracellular mycelium, or intercellular, with or without haustoria. Some fungi, such as the powdery mildews, are almost completely external, putting haustoria into the epidermal cells of the plant. However, some pathogens associate with suscepts for support only, being entirely external. Therefore, the disease relationship is not always a food relationship, and the use of the terms parasite and host should be avoided when discussing disease.

The nature of the injurious activity is varied and extremely complex, so much so that the exact nature of the physiological processes in most cases is unknown. A simplified summary of the possible chemical products involved is the production of toxic metabolic products, enzymes, and hormones. There is also the possibility that the action of the pathogen is mechanical, for example, desiccation and death of suscept tissues due to rupture when the pathogen sporulates, shading effects of epiphytes, and strangulation by epiphytes.

The response of the suscept to the chemical or mechanical stimuli of the pathogen varies in the different disease relationships. The physiological and morphological responses may be quite striking. The toxic metabolic substances and enzymes may cause cell wall and protoplast breakdown, and ultimate death. Gum formation within vessels may result, thus blocking normal water uptake. Hormones or other substances may cause tyloses to form, thus preventing water movement. Hormones may also cause abnormally rapid and increased cell division, callus formation, formation of new periderms, dedifferentiation of matured cells, and premature abscission layer formation.

Respiration, transpiration, food synthesis and translocation of the suscept may be injuriously affected. Since many pathogens take food from the suscept, starvation may also occur, which, if extreme, may result in death.

After invasion and subsequent establishment, new infections on the same plant or on other plants can occur if more inoculum is formed. The inoculum may be produced early or late in the course of the disease. This inoculum, if produced during the current growing season, initiates secondary life cycles. With many fungus pathogens, this inoculum consists of imperfect spores. The abundance of formation of the inoculum and the interrelation of many critical environmental factors determines whether epidemics occur.

Many fungus pathogens have the ability to grow as saprophytes in killed tissues, whereas others, notably the rusts, do not. In many cases, the rusts have already become dormant when the suscept dies. Some fungi do not produce spores on lesions of the living plant, but do so abundantly following death of the tree; for example, Strumella coryneoidea on oak. Furthermore, some fungi produce one type of spore on the living plant and another kind on the dead plant parts or organs. As a response to the less favorable environmental conditions of killed tissues and changing seasons, fungus pathogens respond in characteristic ways, becoming dormant and overwintering in the various ways already mentioned . The life cycle is now complete.

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DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA

INFLUENCE OF LOCATION ON INVASION OF DRY-ICE-KILLED TISSUES ON ITALIAN PRUNE TREES BY NATURALLY DISSEMINATED CYTOSPORA FUNGI[1]

A. W. Helton[2]

Summary

Local areas on stems of 3-year-old Italian prune trees were freeze-killed with blocks of dry ice. One-third of the trees were frozen on the north sides, one-third on the southwest sides, and one-third on the southeast sides. Injured tissues were left exposed. Naturally disseminated Cytospora fungi invaded 77.7% of the test trees, and 14.3% proved virulent.

The data revealed that location of low-temperature-injured tissues on trunks and scaffold branches of prune trees has no effect on Cytospora invasion and/or development of Cytospora canker disease.

INTRODUCTION

Cytospora canker diseases have been serious problems in plum and prune varieties in Idaho since 1951 (3). Pathogenicity characteristics of the causal fungi have been found to vary with isolate and host variety (2). Severity of the Cytospora canker diseases often has been observed to vary with occurrence of predisposing, or worsening, factors in the orchard environment (2, 3).

Winter injury has been notable among the environmental factors observed to affect both initial infection and subsequent development of Cytospora disease; and previous experiments demonstrated that low temperature injury, even in small spots on vigorous trees, provides adequate entry courts for naturally disseminated Cytospora fungi (1).

Winter injury often becomes a localized condition in affected orchards. Sometimes the most severe injury occurs in low spots. Sometimes the injury is localized on affected trees; for example, the sides of trunks and scaffold branches that are exposed to prevailing cold winds, or to daytime heat from the sun that is reflected from snow on the ground. In the northern latitudes these two factors frequently act together and produce most serious damage on the southwest surfaces. Subsequent invasion by Cytospora fungi increases the extent of the damage.

The experiments reported here were carried out to determine whether low temperature tissue-killing, per se, was the determinant environmental factor in natural invasion by Cytospora fungi; or whether the key factor lay in the microclimates associated with different locations on injured trees. That is, on a site where cold winds from one direction were not a problem, would the lower, and more continuously low, temperatures (on the north side) result in more susceptibility of that side to infection; would damaged tissues on the intermediately exposed southeast side be invaded more frequently; or would the freezing and warming due to winter sunlight on the southwest side cause injuries on that side to be invaded most readily.

MATERIALS AND METHODS

Vigorous, well-established, 3-year-old Italian prune trees (Prunus domestica) were selected. The trees occupied a 12-foot grid planting arrangement on a gentle south slope.

A 50-pound block of dry ice was obtained and cut into blocks 2 x 4 x 12 cm with a band saw. These were attached to branches and trunks of the trees on the north, southwest and southeast sides.

Two layers of cheesecloth were placed on one side of each block and the opposite side placed against the stem surface to be frozen. Each block was bound in place with a piece of string in the center and a nurseryman's rubber budding strip at each end. Each block was shaded from the sun with a piece of paper toweling stapled around the stem above the block.

The blocks were attached to stems approximately 3 cm in diameter. Each treatment was replicated 18 times.

Degree of dry ice injury and incidence and progress of natural infection were recorded by the following numerical rating system: 0) No flat-face, that is, no flattened spot formed on the stem surface by failure of cambial growth to take place where dry ice blocks were attached; and

[1]Approved by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 521.
[2]Associate Plant Pathologist, University of Idaho Agricultural Experiment Station.

no infection. 1) Normal healing around dry-ice-injured tissues. 2) Flat-face present, but no infection. 3) Injured area lightly pimpled with pycnidial fructifications of Cytospora fungi. 4) Injured area moderately pimpled. 5) Injured area densely pimpled. 6) Exudation of gum in or near the injured area. 7) A Cytospora canker, with margins expanding beyond the injured area. 8) The injured area infected and parts terminal to it flagging, that is, foliage discolored and drooping. 9) Injured area infected and parts above dead. 10) Injured area infected and canker margins expanding downward. 11) Tree dead of Cytospora canker disease.

RESULTS

The tissue-freezing phase of the experiment was carried out on May 1. The average evaporation time for the dry ice blocks was 2 hours. The average air temperature in the plot during this time was 26° C.

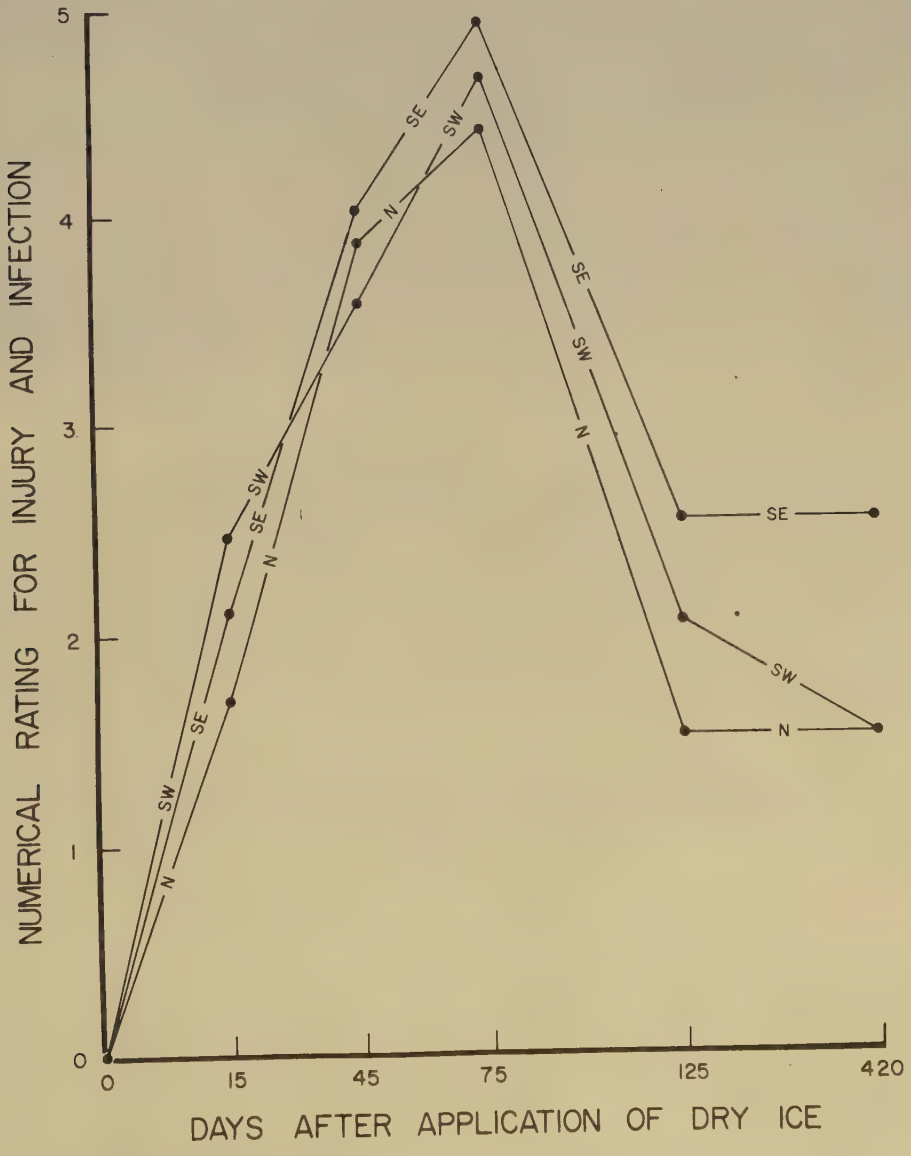

FIGURE 1. Degree of low temperature injury, invasion of frozen tissues by naturally disseminated Cytospora fungi, and virulence of infections when dormant 3-year-old Italian prune trees were damaged locally by dry ice blocks attached to scaffold branches on north, southwest, and southeast sides of test stems. Each value is the average of 18 replicates.

Unfrozen check trees developed no symptoms.

Within 2 weeks after the dry ice blocks were attached there had been sufficient cambial activity that prominent flat-faces had developed where the blocks had been attached (Fig. 1). Within 6 weeks invasion of the frozen tissues by naturally disseminated Cytospora inoculum had occurred commonly throughout the plot.

Pycnidial Cytospora fructifications were observed on 77.7% of the injured trees, and in 14.3% of the cases the invading fungi proved to be virulent, as indicated by development of progressive disease. The average curves of Figure 1 show that location of the freeze-injured tissues had no appreciable effect on initial invasion, whether or not the invading fungi proved to be virulent.

DISCUSSION AND CONCLUSIONS

The decline of the three curves in Figure 1 was caused by the development, after approximately 8 weeks, of normal-appearing callus around many freeze sites in which Cytospora fructifications had been observed. Virulence was sufficiently evident in certain other infections, however, to cause greatest spread among the curves after 125 days in spite of the natural tendency to develop callus and thus lower the arbitrary rating.

The phenomenon of normal-appearing callus around Cytospora cankers frequently has been observed in late season in those commercial orchards where damage from the disease has been severe.

This study demonstrated that the location of artificially freeze-injured tissues on major stems of vigorous Italian prune trees has no effect on whether those tissues will be invaded by naturally disseminated Cytospora fungi. From this it is concluded that invasion, *per se*, by Cytospora fungi, and subsequent development of disease, are not influenced significantly by the different microclimates associated with potential entry courts located in different places on susceptible trees. This evidence verifies orchard observations made in Idaho over the past 10 years.

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IDAHO AGRICULTURAL EXPERIMENT STATION, MOSCOW

INTERRELATIONSHIPS OF RECOGNIZED PRUNUS RINGSPOT SYMPTOMS AND ASSOCIATED SYMPTOMS IN CHERRY TREES[1]

A. W. Helton[2]

Summary

A total of 3542 cherry trees were inspected in detail during a 6-year survey period. All symptoms were recorded in mid-July.

Trees with representative symptoms of the ringspot type, and/or with symptoms known to occur in association with the recognized ringspot symptoms, were selected for transmission studies.

The results of both the survey and the transmission studies indicated that 1) the plum line-pattern virus is not involved in the many chlorotic line symptoms found in cherry trees in Idaho, 2) the prune dwarf factor generally is associated with cherry ringspot inoculum in Idaho but does not always accompany it, and 3) any of a number of ringspot and ringspot-like symptoms observed in orchard trees can be produced in test trees with inoculum from trees showing any of these conditions.

[1]Approved by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 522; study conducted as a part of Western Region Project W-64.

[2]Associate Plant Pathologist, University of Idaho Agricultural Experiment Station.

INTRODUCTION

During the period from 1952 through 1957, selected cherry orchards throughout the fruit-growing districts of Idaho were examined annually on a tree-by-tree basis. All foliar symptoms were recorded on individual orchard maps. This was done to gain information on the reliability of certain symptoms as aids in field diagnosis, and to acquire information on annual differences in symptom expression in trees infected with viruses of the ringspot type (2). The observational data accumulated during the survey period were supplemented by greenhouse and field-plot inoculation studies using virus cultures taken from known-history survey trees.

MATERIALS AND METHODS

All recognized disorders present in 3542 cherry trees were recorded annually, in July, during a 6-year survey period. Progression of individual symptoms in affected trees was not mapped since rate of intra-host movement of viruses was not an objective of the investigation.

The studies were facilitated by a symptom-description code adopted at the beginning of the survey (Table 1).

Greenhouse and plot transmission studies were carried out with inoculum taken from donor trees selected during the survey. Budwood from 12 Bing sweet cherry (Prunus avium) and 3 Montmorency sour cherry (P. cerasus) trees was used in bud-grafting studies in a greenhouse, and 20 Bing cherry and 2 Montmorency cherry trees were sampled for similar tests in a field plot. Budwood was collected from the section of each donor tree where the symptom in question was most prominent. If such budwood was desired during the dormant period, the bud sticks to be taken were marked during the preceding July examination.

Test trees, used as acceptor trees, were provided in two locations. Bing and Lambert sweet cherry (Prunus avium), Montmorency sour cherry, and Mahaleb seedling (P. mahaleb) trees were used in the greenhouse study. Bing and Montmorency cherry, Italian and Weatherspoon prune (P. domestica), and the hybrid plum Shiro were used in the field plot study.

A minimum of three bud sticks were taken from each donor tree on each collection date, and a minimum of three buds were grafted to each of two acceptor trees, in the greenhouse or in the field plot.

RESULTS

Survey Studies: The LPRS, MLRS, TLRS and BL symptoms (Table 1) occurred commonly during the survey period whereas CRS, NRS, and V-L did not (Figs. 1, 2).

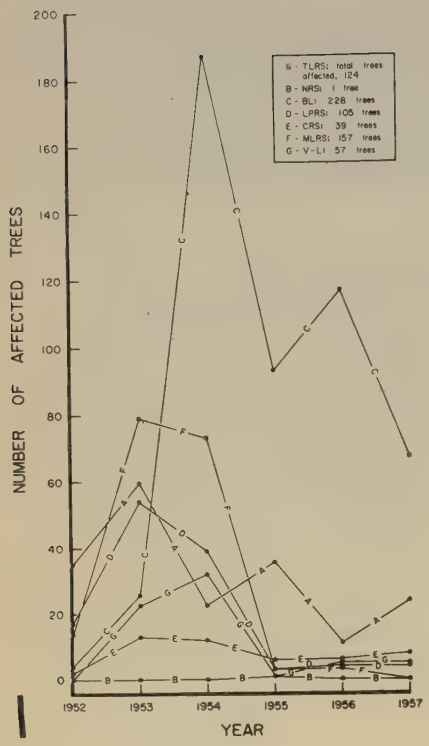
As Figure 1 indicates, the occurrence in Bing sweet cherry trees of LPRS, MLRS and V-L symptoms varied in a marked pattern during the survey period, reaching peaks in 1953-1954. This trend was less prominent for CRS, and it did not hold at all for TLRS and BL. Individual-year differences were much more apparent for TLRS and BL, and the relationship between the annual occurrence of these two conditions appeared in an inverse pattern.

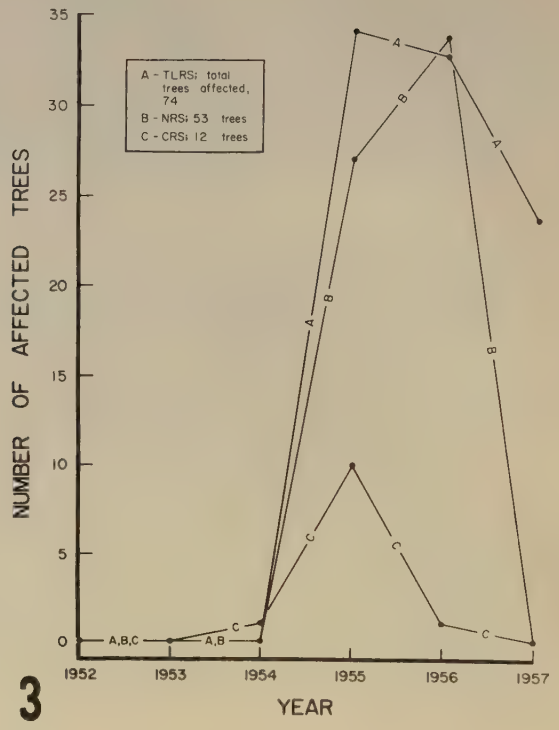
For most of the recorded disorders, recurrence in individual trees was most common on the year or two following initial appearance of the symptoms, but for TLRS recurrence in individual trees was more prolonged (Fig. 2). The recurrence patterns of BL were highly irregular, with individual recurrences more numerous and prolonged even than for TLRS.

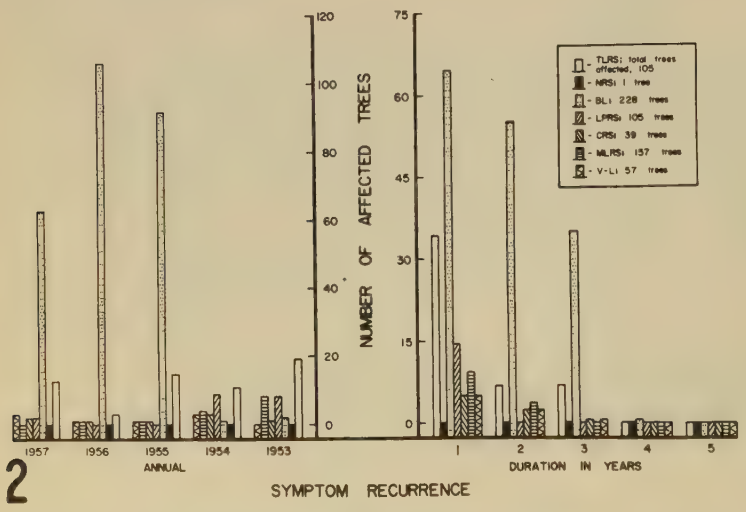
As indicated by the behavior of ringspot symptoms in the one Montmorency sour cherry orchard under detailed survey, such disorders varied considerably in their development on different years (Fig. 3). TLRS again was most persistent in its recurrence in individual trees (Fig. 4), but the duration of this persistence was much less than in the sweet cherry trees. Generally, all the ringspot symptoms found in the sour cherry trees were more transient than similar symptoms in sweet cherry trees.

Ringspot symptoms commonly occurred alone in affected trees, but often they were associated with other such symptoms also (Table 2). Although TLRS occurred alone more often than with other symptoms in sweet cherries, it occurred more often with NRS in sour cherries than it did alone. The CRS symptom sometimes was associated with TLRS in both sweet and sour cherries but not with NRS in either group. LPRS and MLRS occurred with all of the other symptoms in sweet cherries except NRS, which was not found in the sweet cherry orchards under survey except for two cases. As Table 2 indicates, association of these disorders in individual trees was not always concurrent. Sometimes one symptom followed another by a year or more; sometimes it preceded the other. There seemed to be no definite pattern of relationship of the milder symptoms to the severe ones, that is, their association in individual trees was as often one before the other as it was concurrent.

The bent leaf and V-leaf symptoms (4) were encountered throughout the survey period, and as the survey progressed their association with recognized ringspot symptoms became more evident. They were often found alone in individual trees (Table 3), but frequently also they were found in trees that exhibited other symptoms. In fact, their relationships to other symptoms appeared to be no different than the relationships of the other symptoms to each other as described above. They were also found to occur with each other in affected trees, or prior to or following each other.







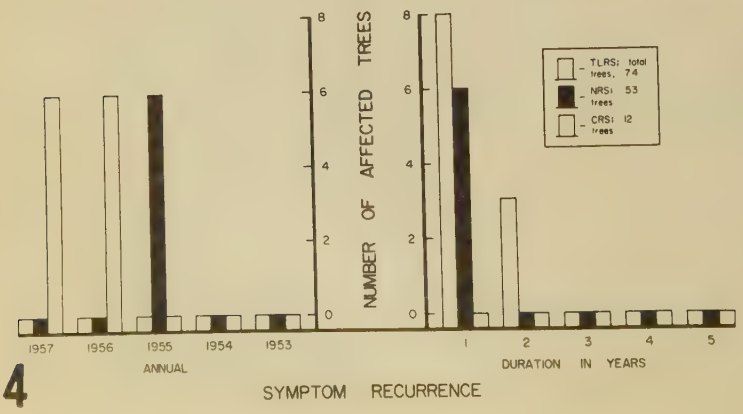


FIGURE 1. Annual incidence of symptoms associated with ringspot viruses in 13 Bing sweet cherry orchards totaling 2669 trees 12 to 35 years old in 1952.

FIGURE 2. Recurrence of symptoms associated with ringspot viruses in individual trees in 13 Bing sweet cherry orchards totaling 2669 trees 12 to 35 years old in 1952.

FIGURE 3. Annual incidence of symptoms associated with ringspot viruses in a sour cherry orchard of 760 7-year-old Montmorency trees and a semi-sweet cherry orchard of 113 trees 8 to 13 years old in 1952.

FIGURE 4. Recurrence of symptoms associated with ringspot viruses in individual trees in a 7-year-old Montomorency sour cherry orchard of 760 trees and a semi-sweet cherry orchard of 113 trees 8 to 13 years old in 1952.

Table 1. Symbolism for symptoms associated with Prunus ringspot viruses and for other conditions occurring in orchard cherry trees and/or in test hosts.

Code	Description
TLRS	Tatter leaf ringspot -- a severe condition of necrotic spotting followed by leaf tattering.
NRS	Necrotic ringspot -- a less severe condition of necrotic leaf spotting, often associated with chlorotic rings, with the necrosis sometimes limited to finer lines and spots within the chlorotic lines.
BL	Bent leaf -- a less severe condition in which the middle area of one side of the affected leaf is prominently chlorotic and the leaf bends strongly around the chlorotic area without curling or twisting.
LPRS	Line-pattern ringspot -- a less severe condition of irregular chlorotic lines ranging from bright to pale yellow, generally without necrosis, sometimes associated with chlorotic rings.
CRS	Chlorotic ringspot -- a less severe condition in which pale to prominently chlorotic rings up to approximately 1/2 inch diameter form on undistorted leaves in single or concentric fashion.
MLRS	Mottle-leaf ringspot -- a less severe condition in which pale chlorotic mottling develops over leaf surfaces, often preceding or accompanying chlorotic rings, but transient and not due to mottle leaf viruses.
V-L	V-leaf -- a very mild and unusually constant form of line chlorosis in which a rough "V" is formed with the apex of the V in the midrib area below the leaf tip and the sides ending in the leaf margins near the middle of the leaf, the sides often zig-zagging toward the midrib in the interveinal areas, and suggestive of a nutritional condition.
PD	Prune dwarf -- a virus-induced stunting, strap-shaping and roughening of the leaves of prune trees.
GRM	Green ring mottle -- a virus-induced condition of semi-sweet and sour cherry leaves in which prominent greenish rings of approximately 1/2 inch diameter form on otherwise yellow leaves.
RM	Rusty mottle -- a virus-induced condition of sweet cherry trees in which affected trees are stunted, affected leaves are prominently mottled with pale green and rusty-yellow, considerable defoliation occurs, and the condition is evident at nearly all times that foliage is present.
RugM	Rugose mosaic -- a virus-induced condition of sweet cherry trees in which prominent chloroses occur in sub-terminal portions of affected leaves and twisting, curling upward and inward over chlorotic areas, and other distortion is common.

Table 2. Patterns of occurrence of symptoms of the ringspot type in cherry trees.

	Occurrence in individual trees			
Symptom	times alone	times with other individual symptoms	times before other individual symptoms	times after other individual symptoms
Sweet cherry				
TLRS	147	LPRS-1; CRS-3; MLRS-4	NRS-1[b], 1[c], CRS-2[a]; MLRS-4[a], 5[b]	NRS-2[a]; CRS-1[a], 1[d]; MLRS-4[a], 1[b], 5[c], 2[d]
NRS	0	0	0	TLRS-2[a]
LPRS	107	---	---	---
CRS	32	LPRS-6; MLRS-1	LPRS-1[a]	LPRS-3[a], 1[c]; MLRS-2[a], 1[b]; TLRS-1[a]; NRS-1[a]; TLRS-1[b], 1[c]
MLRS	16	LPRS-4	MLRS-1[a]	LPRS-1[a]
Sour cherry				
TLRS	31	NRS-47; CRS-4	NRS-3[a]; CRS-2[a]	NRS-2[a], 3[b]; CRS-1[a]
NRS	12	---	---	---
CRS	6	---	---	---

[a]One year before or after the symptom at the left of the table.
[b]Two years before or after the symptom at the left of the table.
[c]Three years before or after the symptom at the left of the table.
[d]Four years before or after the symptom at the left of the table.

Table 3. Occurrence of bent leaf (BL) and V-leaf (V-L), probably ringspot variations, in relation to occurrence of recognized ringspot symptoms in sweet cherry trees.

	Occurrence in individual trees			
Symptom	times alone	times with other individual symptoms	times before other individual symptoms	times after other individual symptoms
BL	275	TLRS-6; MLRS-58; LPRS-23; CRS-3; VL-23	TLRS-6[a], 3[b], 2[c]; LPRS-2[a], 3[c]; CRS-1[a], 4b, 1[c]; MLRS-1[a]; VL-2[a], 1[c]	TLRS-3[a], 4[b], 4[c], 4[d], 1e; MLRS-1[a], 33[b], 19[c], 2[d]; LPRS-5[a], 18[b], 10[c], 2[d]; VL-2[a], 14[b], 4[c]
V-L	16	TLRS-1; LPRS-19; CRS-3	TLRS-1[b], 1[c]; NRS-1[a]	TLRS-1[a], 1[b]; LPRS-5[a]; CRS-1[d]

[a]One year before or after the symptom at the left of the table.
[b]Two years before or after the symptom at the left of the table.
[c]Three years before or after the symptom at the left of the table.
[d]Four years before or after the symptom at the left of the table.
[e]Five years before or after the symptom at the left of the table.

Table 4. Interrelationship of ringspot and associated symptoms as indicated by field-run inoculum in virus-free greenhouse trees over a 2-year period.

Source tree		Symptoms in test trees[a]			
number	symptoms	Bing sweet cherry	Lambert sweet cherry	Montmorency sour cherry	Mahaleb cherry
Sweet cherry					
9	TLRS	CRS; TLRS	Extreme die-back	Necrotic petal spots	---
13	NRS	Bud gummosis; TLRS; RS shock death	---	---	CRS; death
14	MLRS	---	MLRS; BL; Die-back	---	MLRS
15	TLRS	---	MLRS	---	LPRS
16	CRS	TLRS; RS shock death	MLRS; CRS	---	MLRS
17	V-L	MLRS; die-back	MLRS; CRS	---	MLRS; LPRS
18	BL	MLRS; NRS; die-back	---	---	CRS
19	LPRS; CRS	MLRS; die-back	---	---	MLRS; distortion
20	LPRS	---	---	Bud gummosis	MLRS; distortion
21	LPRS	LPRS; RM	---	---	NRS
22	TLRS	TLRS; RS shock death	---	---	MLRS; CRS; distortion
23	CRS; TLRS	---	---	Stunting	MLRS; CRS
Sour and semi-sweet cherry					
1	GRM	---	--- ---	Necr. petal spots	CRS
2	NRS; TLRS	---	---	Severe stunt	MLRS
3	NRS; TLRS	---	---	Severe stunt	MLRS

[a]Plural symptoms generally concurrent but mild forms sometimes preceding severe forms in order indicated; for symptom definitions see Table 1.

Table 5. Interrelationship of ringspot and associated symptoms as indicated by field-run inoculum in virus-free plot trees over a 3-year period.

Source tree		Symptoms in test trees[a]				
number	symptoms	Bing sweet cherry	Montmorency sour cherry	Italian prune	Weatherspoon prune	Shiro plum
Sweet cherry						
1	CRS	LPRS; TLRS	---	TLRS; PD	PD	0
2	CRS	LPRS	---	0	PD	0
3	LPRS	MLRS	---	---	0	0
4	NRS	TLRS	---	---	PD	0
5	TLRS; RugM	TLRS; RM	---	---	0	---
6	TLRS	MLRS	---	---	PD	0
7	CRS	0	---	0	PD	0
8	NRS	---	---	---	PD	0
9	TLRS	TLRS	---	---	---	---
10	LPRS	MLRS	0	---	0	---
11	V-L	TLRS	---	---	0	0
12	BL	LPRS	---	---	---	---
13	NRS	TLRS	pale foliage	---	0	---
15	TLRS	Death	---	---	---	---
17	V-L	0	---	CRS; TLRS	---	---
18	BL	0	---	---	---	---
19	CRS; LPRS	0	---	0	---	---
21	LPRS	Stem necr.	---	---	---	---
22	TLRS	TLRS; stem necr.	---	---	---	---
23	CRS; TLRS	0	0	CRS; TLRS	---	---
Sour cherry						
2	NRS; TLRS	---	CRS	---	---	---
3	NRS; TLRS	---	0	---	---	---

[a]Plural symptoms generally concurrent but mild forms sometimes preceding severe forms in order indicated; for symptom definitions see Table 1.

Inoculation Studies: The 15 source trees selected for bud-grafting experiments in a greenhouse represented the various ringspot and ringspot-like symptoms, as indicated in Table 4. The results demonstrated the same interrelationships of the ringspot symptoms that had been observed during the orchard surveys.

In like manner, donor budwood was variously grafted to acceptor trees in a field plot as indicated in Table 5. The Shiro plum was used to reveal whether any of the line-pattern chloroses of common occurrence in the orchard were related to the line-pattern virus of plums (2), for which Shiro is an excellent indicator. All results were negative on Shiro plum. Inoculations to the prune varieties were made to reveal whether the prune dwarf factor (5) was carried in the cherry ringspot inoculum. Frequent positive results (Table 5) demonstrated that it was.

The Bing and Montmorency inoculations were made, as in the greenhouse study, to reveal whether the symptoms observed on the donor trees could be reproduced with field-run inoculum or whether other of the ringspot symptoms would result.

As Table 5 shows, the same interrelationships of the symptoms resulted in the inoculated trees as developed in the greenhouse and as had been observed in the surveyed orchards.

DISCUSSION AND CONCLUSIONS

The results demonstrated that the plum line-pattern virus is not involved in the chlorotic line symptoms associated with the occurrence of ringspot viruses in the source trees tested. Therefore, it is unlikely to be of common occurrence in Idaho cherry orchards.

The data confirm previous reports (1, 7) that the prune dwarf factor is of common occurrence in orchard cherry trees that exhibit various of the ringspot symptoms. However, since CRS symptoms similar to those seen in the source trees occasionally were produced in inoculated prune trees, without development of PD symptoms, the results indicate that the prune dwarf factor is not present in all field-run ringspot inoculum, as had been suggested (6).

The distinct annual fluctuations observed in the symptoms of infected orchard trees suggests a prominent environmental effect on symptom expression; for example, not only were 1953 and 1954 relatively high-incidence years, but when TLRS symptoms were abundant BL symptoms decreased and vice versa (Fig. 1). While "shock" symptoms cannot be ruled out as a partial explanation for certain peak-year effects, for example, in the Montmorency orchard in 1955-1956, (Fig. 3), the fact that the trees were at least 10 to 11 years old, and usually much older, on the peak-years points also to an environmental factor. Ringspot indexing of orchard trees on Shiro-fugen (P. serrulata) in Idaho has for many years indicated that earlier infection is more common than these peak-year results indicate.

The overall evidence is circumstantial in nature, but it suggests that the various ringspot symptoms to be found in bearing cherry trees are but variations induced by strains of a parent, broad-spectrum ringspot virus type. It suggests also, therefore, that field diagnoses (4) of ringspot virus infection can be reasonably reliable despite the obviously wide variation in symptom expression and despite the latent nature of the ringspot viruses involved.

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IDAHO AGRICULTURAL EXPERIMENT STATION, MOSCOW

DISEASES OF FORAGE GRASSES AND LEGUMES PREVIOUSLY UNREPORTED FROM NEW HAMPSHIRE[1]

R. A. Kilpatrick[2]

Summary

Surveys of diseases occurring on forage grasses and legumes in New Hampshire have been made at frequent intervals during the growing seasons of the past 5 years. Eighteen diseases, 8 on forage grasses and 10 on legumes, are reported for the first time from this area.

The diseases occurring on forage grasses and legumes each year in New Hampshire are, in general, relatively constant, but there are annual variations. Disease severity varies from year to year, and from season to season. Since earlier references (1, 2, 3, 4) have listed some of the diseases occurring on forage grasses and legumes in New Hampshire, this supplements the earlier reports.

GRASS HOSTS:

1. Barnyard grass (Echinochloa crus-galli)
   Node smut (Ustilago crus-galli Tracy & Earle) is generally found during the latter part of the growing season, being common in some areas.
2. Brome (Bromus inermis)
   Rhynchosporium scald (Rhynchosporium secalis (Oud.) J. J. Davis) is found on brome each year, but it is of minor importance in New Hampshire.
3. Oats (Avena sativa)
   Two diseases have been associated with this host: Helminthosporium leaf spot (Helminthosporium sp.) and leaf rust (Puccinia recondita Rob. ex. Desm.). Both have been of minor importance.
4. Orchardgrass (Dactylis glomerata)
   Brown stripe (Scolecotrichum graminis Fckl.) is the primary disease on this host in the State. At present, it is not of much concern. Differences in host susceptibility are apparent.
5. Quackgrass (Agropyron repens)
   Helminthosporium leaf spot (Helminthosporium tritici-repentis Died.) is found in most years, but it appears to be of minor importance.
   Tar spot (Phyllachora graminis (Pers. ex Fr.) Fckl.) has been found in isolated areas each year.
6. Timothy (Phleum pratense)
   A Helminthosporium leaf spot (Helminthosporium sp.), similar to that caused by H. giganteum Heald & Wolf, was found in 1961 at Northwood, N. H.

LEGUMINOUS HOSTS:

1. Alfalfa (Medicago sativa)
   Yellow leaf blotch (Pseudopeziza jonesii Nannf.) was found in the spring of 1958 at Northwood, N. H. for the first and only time.
2. White sweet clover (Melilotus alba)
   Stagonospora leaf spot (Stagonospora meliloti (Lasch.) Petr.) (Leptosphaeria pratensis Sacc. & Briard) was abundant on

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[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and Departments of Botany and Agronomy, New Hampshire Agricultural Experiment Station, Durham. Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 281.

[2]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

volunteer plants growing along the roadside in northern New Hampshire (Colebrook) in July 1961.
Pseudopeziza leaf spot (Pseudopeziza sp. (P. meliloti Syd. ?)) was found on sweet clover plants at Colebrook in July 1961. Lesions and apothecia were numerous on some plants. This disease has apparently been overlooked as symptoms are abundant on clovers in this area each year.

3. Alsike clover (Trifolium hybridum)
   Cercospora leaf spot (Cercospora zebrina Pass.) is found on alsike clover plants each year. It is not so severe as rust, sooty blotch, mildew or Pseudopeziza leaf spot.
4. Hop clovers (Trifolium species)
   These plants are found throughout New Hampshire. Leaf spots found include: Botrytis leaf spot (Botrytis cinerea Pers. ex Fr.), Cercospora leaf spot (Cercospora zebrina), and powdery mildew (Erysiphe polygoni DC.). Fungi isolated from leaves and stems include Phoma sp., Alternaria sp., and Cladosporium sp.
5. Rabbit-foot clover (Trifolium arvense)
   This clover is found growing along the roadsides throughout the State. Two diseases have been observed on this host, namely Cercospora leaf spot (Cercospora zebrina) and Botrytis leaf spot (Botrytis cinerea). The latter disease was found in July 1961.
6. White clover (Trifolium repens)
   Botrytis leaf spot (Botrytis cinerea) was found near Walpole, N. H. in July 1961. Because of the resemblance to other clover leaf spots (Curvularia, Stagonospora, northern anthracnose, and bacterial leafspot) isolations were necessary to confirm the identity of the pathogen which infected 25 to 40% of the leaflets.

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DEPARTMENTS OF BOTANY AND AGRONOMY, UNIVERSITY OF NEW HAMPSHIRE, DURHAM, NEW HAMPSHIRE

PHYSIOLOGICAL STUDIES ON HOST-PARASITE RELATIONSHIP OF THE ROOT-KNOT NEMATODE, MELOIDOGYNE JAVANICA

Bakir A. Oteifa and Dawood M. Elgindi

Attraction of nematode larvae to growing plants is an important physiological mechanism that depends largely on the type of plant and nature of the chemical stimuli which diffuse from their active growing roots.

In connection with a series of experiments conducted in 1959 on the physiological behavior of the root-knot nematode, Meloidogyne javanica (Treub, 1885) Chitwood, 1949, two tests were carried out to demonstrate quantitatively the percentage larval attraction to different sources of stimuli. The method of recording the attraction index in both tests was essentially that described by Wieser (2).

The first test was designed to investigate the effect of seedling root sizes of both a host and a non-host plant on larval attraction. Seeds of tomato and barley were allowed to germinate on wet filter papers in Petri dishes at room temperature. Upon germination six different root sizes were selected. For each root size three uniform seedlings were used as replicates. Figures 1 and 2 illustrate the percentage attraction of larvae of Meloidogyne javanica when subjected to different root sizes of both tomato and barley seedlings. Apparently barley seedlings are not attractive to larvae. On the other hand tomato seedlings exert a strong attraction. The maximum attraction occurred when the root size was between 10 and 15 mm; shorter and longer roots were less attractive. This indicates that larval attraction depends on plant specificity, and the process of larval attraction to growing plants is connected with the metabolizing activities during the early stages of plant growth and development.

On the assumption that amino acids are diffused from roots of actively growing plants (1), the second test was designed to test larval attraction of 14 amino acids. Amino acids used were, glycine, arginine, isoleucine, lysine, histidine, phenylalanine, methionine, tryptophane, tyrosine, glutamine, cystine, leucine, asparagine and alanine. These were prepared by adding 5 mg to 5 ml of steam sterilized 2% aqueous agar solution in a test tube. The contents of each tube were then thoroughly mixed and poured into a capsule. After cooling, the agar was divided into three disc-shaped sectors. For each amino acid the three discs prepared were used as replicates.

The different amino acids used had different attraction values (Fig. 3). Tyrosine, in particular, had a maximum attraction index, while the other amino acids were either neutral or repellent to larvae. It is suggested that the materials that diffuse from roots of host plants probably contain a considerable amount of tyrosine during the early stages of plant growth.

The results also suggest that there is a stage early in the growth of seedlings when they are most likely to be attacked by root-knot nematodes, and that the probability of attack diminishes as they become larger. From the practical standpoint, this implies that seedlings grown in a nematode-free seedbed and transplanted into a nematode-infested field after passing the critical stage would be less liable to infection than plants grown from seed in the field.

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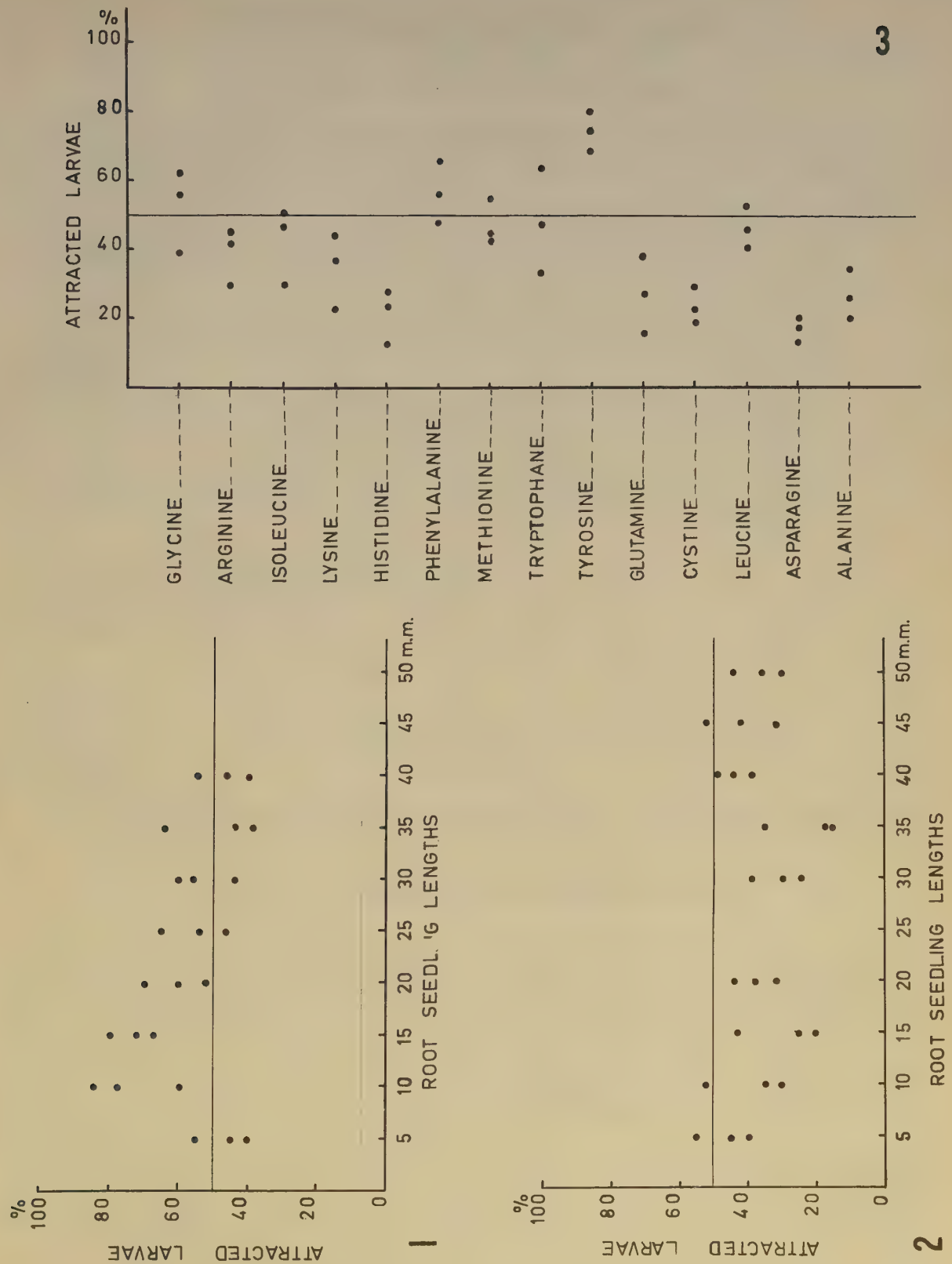
FIGURE 1. Percentages of larvae of Meloidogyne javanica found in the vicinity of tomato roots after 24 hours.

FIGURE 2. Percentages of larvae of Meloidogyne javanica found in the vicinity of barley roots after 24 hours.

FIGURE 3. Percentages of larvae of Meloidogyne javanica found in the vicinity of agar discs containing amino acids after 24 hours.



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PLANT PROTECTION DEPARTMENT, FACULTY OF AGRICULTURE,
CAIRO UNIVERSITY, U. A. R.

A CRITICAL METHOD FOR EVALUATING TOLERANT LEVELS IN NEMATIZED HOST PLANTS

Bakir A. Oteifa and Dawood M. Elgindi

A mathematical equation derived from interactions between plant growth, nematode population density, and environmental resistant factors can be taken as a precise tolerance measurement for making critical comparisons between nematized host plants. The procedure is as follows:

a. Determination of percentage growth.
   Plant growth of infected plants as measured by either yield or dry top weight is expressed as percentage of the potential growth. The potential growth is that of the uninfected control.

b. Determination of nematode population density.
   This is the logarithm of the number of egg masses per gram of dry root.

c. Determination of the logarithm of the number of egg masses when growth is reduced by 50%. (Log n/50).
   A regression coefficient analysis is carried out using the data of steps a and b. From the slope of the line obtained, one can determine the logarithm of the number of nematodes which causes 50% growth reduction.

d. Determination of the environmental resistant factor (E. R. F.).
   Since nematode populations are always in a dynamic state, their rate of population buildup as well as their rate of reproduction are governed by an environmental resistant factor calculated from the formula:

$$\text{Rate of population increase (R. P. I.)} = \frac{\text{Rate of reproduction (R. R.)}}{\text{Environmental Resistant Factor (E. R. F.)}}$$

Rate of population increase can be determined by means of the following formula:

$$\text{R. P. I.} = \frac{P_2 - P_1}{P_1 (t_2 - t_1)} \quad \text{where}$$

P_1 = Initial number of nematodes used in inoculum.
P_2 = Final number of nematodes produced.
$(t_2 - t_1)$ = Time elapsed.

Rate of reproduction can be determined by means of the following formula:

$$\text{R. R.} = \frac{\text{No. of larvae produced}}{\text{No. of mature females produced}} \quad \text{divided by unit root wt. x 100}$$

The E. R. F. can thus be computed as $\frac{\text{R. R.}}{\text{R. P. I.}}$

e. Values obtained from steps c and d are then used in the following mathematical equation to calculate the tolerance level:

$$\text{T. L.} = \sqrt{(\log n/50^2 \pm (\text{E. R. F.})^2} \quad \text{where}$$

T. L. = Tolerance level
Log n/50 = Log. No. of egg masses when growth is reduced 50%.
E. R. F. = Environmental resistant factor.

Table 1.

Treatments (host plants)	Log n/50	E. R. F.
5	3.29	32.4
6	5.80	16.6
8	2.90	13.5

Values of both log n/50 and E. R. F. presented in Table 1 are plotted in Figure 1.

FIGURE 1.

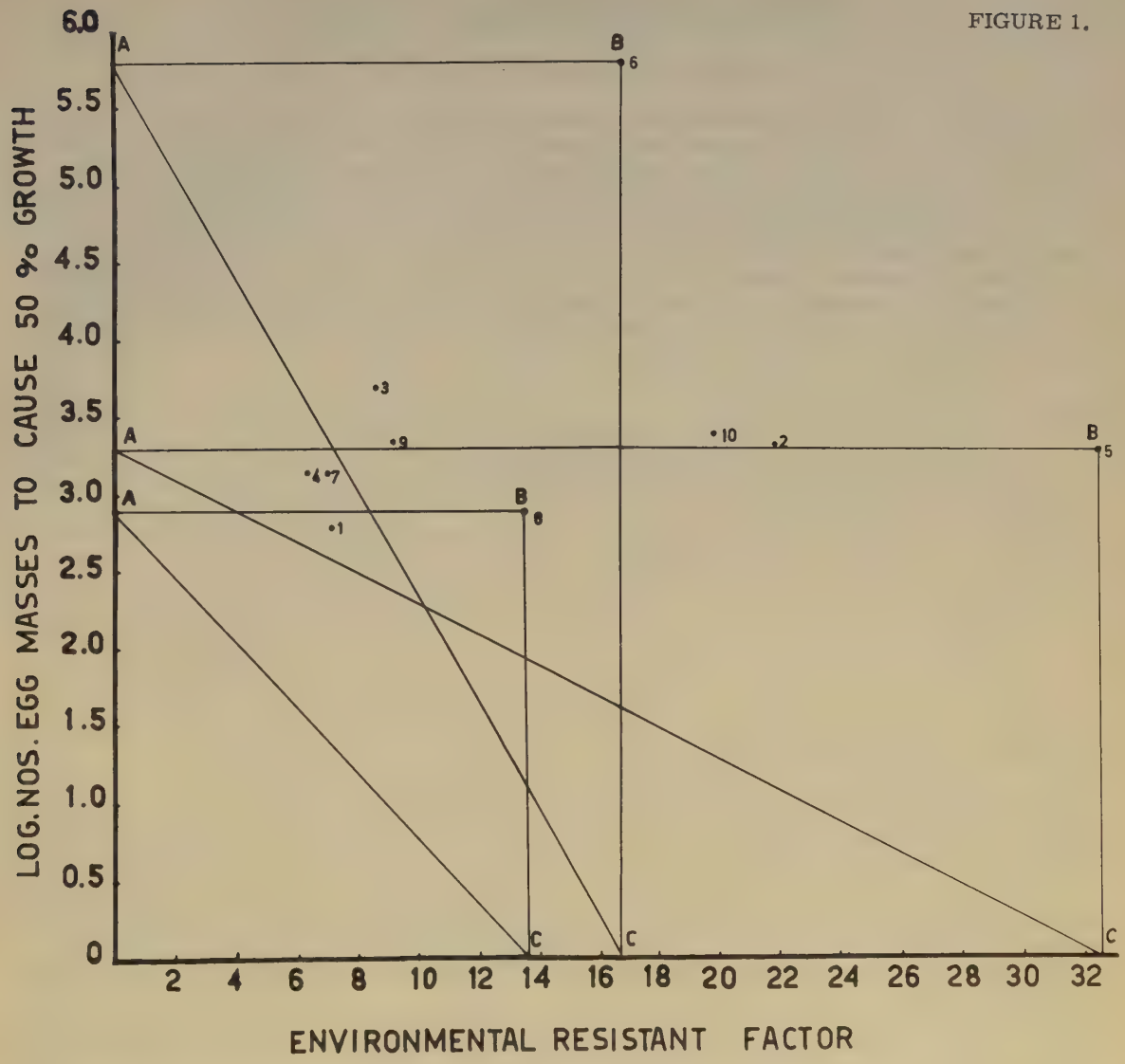

Figure 1 shows that for each treatment the interaction of values results in the formation of a triangle A B C in which the line A C equals $\sqrt{(AB)^2 + (BC)^2}$. The length of A C thus represents the tolerance level. The higher this value is the higher the degree of tolerance.

This approach towards formulating a value defining the interaction between plant growth and nematode population will undoubtedly be of great help to plant breeders in evaluating the degree of host tolerance.

PLANT PROTECTION DEPARTMENT, FACULTY OF AGRICULTURE,
CAIRO UNIVERSITY, U. A. R.

SIGNIFICANCE OF POTASSIUM FERTILIZATION IN NEMATODE INFESTED COTTON FIELDS

Bakir A. Oteifa and Khalil A. Diab

There is no consistent agreement in literature pertaining to the value of applying different levels of inorganic fertilizers to nematode infested soils. Accordingly, a project was initiated in an attempt to alleviate nematode damage by proper host fertilization balance.

Cotton, Gossypium barbadense var. Ashmouni, was grown in an experimental area which was previously sampled in order to assure the presence and uniformity of infestation by plant-parasitic nematodes.

Fertilizers broadcast by hand, after thinning of seedlings, were ammonium nitrate containing 20.5% N, superphosphate containing 15.5% P_2O_5 and sulfate of potash containing 48% K_2O. Nine treatments, each replicated four times as shown in Table 1, were arranged in a randomized block design.

Table 1.

Fertilizer trials (kilograms/feddan[a])		
N	P	K
0	0	0
75	0	0
150	0	0
0	100	0
0	200	0
0	0	100
0	0	200
75	100	100
150	200	200

[a]A feddan is 4200 square meters.

A total of 180 soil samples were taken at five times from the time of application to crop maturity. Each sample consisted of 1 pint of soil from the rhizosphere. After nematodes were extracted by a modification of the Christie and Perry method[1], they were counted and identified.

Nematodes of the genera Tylenchorhynchus and Pratylenchus were the predominant types recovered from the soil samples. The numbers of Pratylenchus, however, are not indicative of their total numbers in the field. They are endoparasitic in the root tissues; consequently their percentage recovery from soil samples are low compared with Tylenchorhynchus, which is an ectoparasite.

Statistical analysis on the numbers of recovered nematodes reveals that the reaction of fertilizers on nematode population is of significance only for a period of about 2 months from the time of fertilizer application.

Evaluation of fertilizer levels was based on a correlation between crop response and degree of soil infestation by nematodes, employing the tolerant level equation proposed by Oteifa and Elgindi[2]. The results indicated that plants under different fertilizer treatments differ quantitatively in their tolerance to nematode infestation. In general, fertilizers increase the reproductive rate of nematodes; however, the degree of plant tolerance of their damage depends upon the types and levels of fertilizers used. Unfertilized plants show the least degree of tolerance despite the low reproductive rate of the nematodes, while fertilized plants show better tolerance levels despite the increase in nematode reproductive rate. In this respect, an increase in potassium, whether alone or in combination with other elements, increases tolerance of host plants, while allowing for the highest rate of nematode reproduction.

This suggests that nematodes are associated in some manner with the available potassium of the host. When plants are deficient in potassium nematode damage is increased despite their low rate of reproduction. On the other hand, when surplus amounts of potassium are used, nematode damage is markedly reduced, even though the rate of reproduction is high.

NATIONAL RESEARCH CENTRE, NEMATOLOGICAL INVESTIGATIONS, SOIL BIOLOGY UNIT, EGYPT, U. A. R.

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SEED DISINFESTATION WITH PHENACRIDANE CHLORIDE, A BROAD-SPECTRUM BACTERICIDE-FUNGICIDE

QK 40 .548

B. C. Smale[1], M. D. Montgillion[1], and E. H. Toole[2]

Summary

Phenacridane chloride (PAC) effectively disinfested tomato, eggplant, and pepper seeds without reducing the germination rate or total germination. The broad-spectrum antibacterial, antifungal compound was effective in disinfestation of tomato seeds when applied as a soak, dip or spray. Germination of tomato and eggplant seeds treated with PAC and then stored for 1 month in open or sealed containers was not adversely affected. This yellow water-soluble compound was readily absorbed and tightly bound by the seed coats but did not penetrate deeper.

INTRODUCTION

The antimicrobial properties of phenacridane chloride (9-(p-n-hexyloxyphenyl)-10-methyl-acridinium chloride)[3] have been observed in vitro with bacteria and fungi saprophytic and parasitic on animals (2, 3). Recently, the protectant properties of the compound were demonstrated for several economically important plant diseases (1, 4). In the course of investigations involving phenacridane chloride (PAC), it was noted that different plant surfaces such as roots, flower parts, and seed coats were easily stained by the yellow compound and that the stain was not removed readily with water. This persistence of the compound led to research described herein on the efficacy of phenacridane chloride as a disinfestant for tomato, eggplant, and pepper seeds.

MATERIALS AND METHODS

Untreated tomato seeds, variety Beefsteak[4], harvested in 1960 were used in this study. Seeds of the same variety and from the same lot but treated commercially with a fungicide containing 50% thiram were used for comparison. Untreated eggplant seeds, variety Long Purple, and untreated pepper seeds, variety All Big, were also used in evaluation of phenacridane chloride disinfestant properties.

The freshly harvested, undried tomato seeds were extracted from consumer fruit several hours before use. These seeds, prepared without fermentation, were hand-rubbed against a fine wire screen under running water until the amount of gelatinous sheath on the seeds was approximately the same as on seeds marketed commercially.

Solutions of PAC from 8000 through 31 ppm prepared with distilled water in one-half serial dilutions were applied to seeds in most cases by dip (10-second immersion) or by 5-minute soak. Controls dipped or soaked in distilled water were similarly prepared. Seeds were spread on paper toweling in a laboratory at 22° to 25° C and 50 to 60% relative humidity and allowed to dry for at least 72 hours before germination-disinfestation studies.

A laboratory procedure designed to simulate the commercial method of mist seed treatment was devised. An all-glass laboratory atomizer of the type used for application of indicators to paper chromatograms delivered sufficient spray in 5 seconds to cover 5 g of the tomato seeds uniformly. After spray application of distilled water or PAC solutions of 1000, 2000, 4000, or 8000 ppm, seeds were dried with a flow of warm air for 4 hours before disinfestation experiments were made.

Seed-germination studies after PAC treatments involved four to eight replicates of 100 seeds each for tomato and eggplant and 100 seeds not replicated for pepper[5]. Seeds placed between

[1]Plant Pathologist and Agricultural Research Technician, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.
[2]Collaborator, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, and Consultant, Asgrow Seed Company, Twin Falls, Idaho.
[3]Compound supplied by Abbott Laboratories, North Chicago, Illinois.
[4]Seeds were supplied by the Asgrow Seed Company, New Haven, Connecticut.
[5]Germination studies were carried out in accordance with the rules and regulations of Federal Seed Act as published in Service and Regulatory Announcements No. 156, June 1960.

wet blotters were incubated at high humidity for 14 days at temperatures alternating daily between 20° (16 hours) and 30° C (8 hours). Percentage germination was based on the number of normal seedlings that developed during the 14-day period. The number and length of seedlings that developed by the end of 5, 6, and 7 days for tomato, eggplant, and pepper, respectively, reflected the rate of germination.

The percentage seed infestation was obtained by distributing seeds (four replicates of 50 each) over the surface of sterile nutrient agar[6] contained in Petri dishes and maintaining the dishes for 5 days at 28° C. At 1-, 2-, and 5-day intervals, counts were made of the number of seeds with which bacterial or fungal colonies were associated. Evaluation of seeds at the three intervals was necessary, particularly in the case of untreated seeds, since fungal growth arising from infested seeds, while isolated and distinct at the 1- and 2-day intervals, resulted frequently in such extensive mycelial growth after 5 days that bacterial infestation of other seeds in the planted sample was obscured.

RESULTS AND DISCUSSION

Efficacy of Soak, Dip, and Spray Treatment: Phenacridane chloride soak treatment effectively disinfested tomato seeds without reduction of the rate of germination or of the total germination (Table 1, Figs. 1, 2). Immersion of seeds for 5 minutes in 500, 1000, or 2000 ppm solutions of the compound reduced bacterial infestations associated with tomato seeds to 3, 1, and 0%, respectively. These marked reductions are in contrast to the 28% bacterial infestation associated with seeds treated with the thiram fungicide.

Although no adverse effects on germination resulted from PAC-soak treatment, necrotic areas on the hypocotyl-radicle in an average of 4% of the seedlings occurred following germination of seeds soaked in 4000 ppm solutions. The average frequency of necrosis as a result of the 2000 ppm treatment was only 1% whereas no necrosis resulted with the 1000 ppm level of treatment. Frequent examination of the germinating seedlings showed that necrosis did not result from injury to the embryo but occurred when the elongating hypocotyl-radicle came in contact with the high concentrations of PAC on the seed coats.

The increased rate of germination associated with tomato seeds soaked for 5 minutes in distilled water in contrast to the rate of those not given the water treatment also occurred when aqueous phenacridane chloride was used in place of distilled water. The size of seedlings resulting from PAC-treated seeds, water-treated seeds, and untreated seeds at the end of 5 days reflects this rate increase (Fig. 3).

Two thousand ppm PAC was required for complete disinfestation of tomato seeds by dip treatment (Table 4). Seed treatment with 2000 ppm PAC by the dip method (which is a more suitable method of application where large quantities of seeds are involved) was almost as effective as soak treatment with 1000 ppm PAC. As would be expected, dipping seeds in concentrations of 8000 ppm PAC or less did not reduce the rate or total germination.

PAC applied as a spray to tomato seeds resulted in uniform coverage and did not adversely affect germination. Complete disinfestation of the spray-treated seeds resulted following applications of 8000 and 4000 ppm. Two percent of the seeds receiving 1000 and 2000 ppm spray treatment were not disinfested. These results indicate clearly that seed disinfestation through spray or mist application of phenacridane chloride should be easily accomplished. Although other factors may be involved, apparently the high surfactant property of PAC resulting in uniform coverage of the seeds and the marked antimicrobial properties of the compound are important in the spray method of seed treatment.

Complete disinfestation of pepper seeds without adverse effects on germination was accomplished with 5-minute soak treatment in a 1000 ppm solution of PAC (Table 1). Since these seeds were as heavily infested with fungi as with bacteria, the antifungal properties of phenacridane chloride were also clearly demonstrated.

Disinfestation of eggplant seeds was accomplished by a 5-minute soak in 500 ppm solutions of PAC (Table 1). Although soak treatment in 1000 ppm solutions of PAC resulted in a slight retardation of germination rate initially, the retardation effect had disappeared at the end of 11 days.

The germination of tomato and eggplant seeds treated with PAC and then stored for 1 month in open or sealed containers was not adversely affected (Table 3). Similarly, germination of the PAC-treated tomato seeds was not inhibited even after 3 months' storage in sealed containers.

[6]Medium composition: proteose-peptone, 5 g; yeast extract, 3 g; beef extract, 1.5 g; sodium chloride, 3.5 g; dextrose, 1 g; KH_2PO_4, 1.32 g; K_2HPO_4, 3.68g; water, 1000 ml.

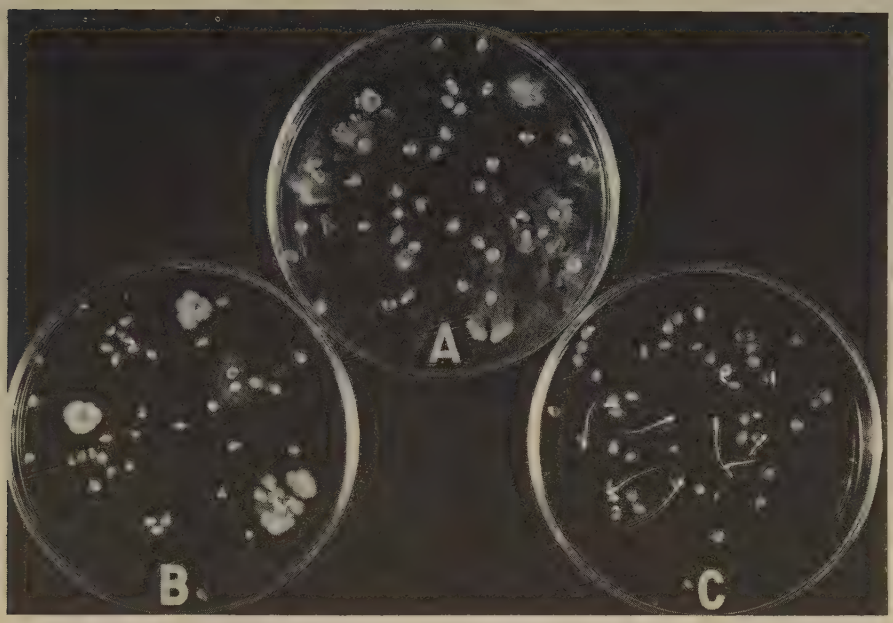

FIGURE 1. Relative infestation of Beefsteak tomato seeds 5 days after treatment. Fifty seeds were placed aseptically on bacteriological agar medium after treatment by A -- 5-minute soak in water, B -- thiram-containing seed protectant dust, and C -- 5-minute soak in a 1000 ppm solution of phenacridane chloride.

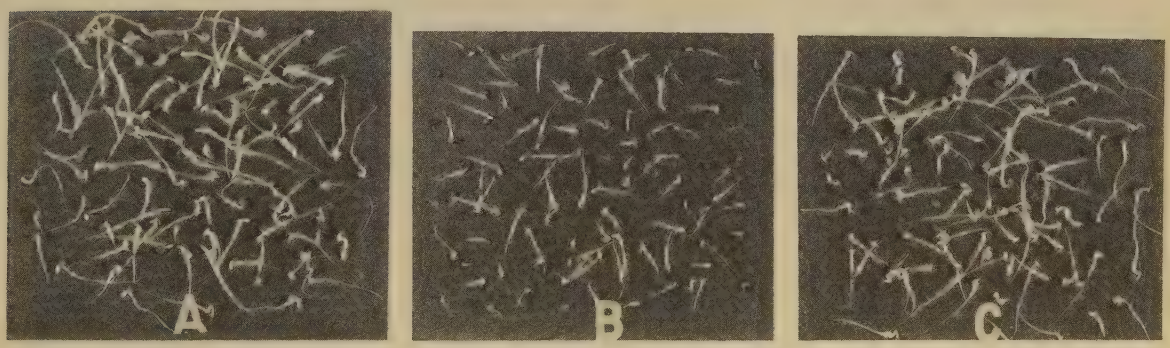

FIGURE 2. Relative germination of Beefsteak tomato seeds at the end of 5 days' incubation. A -- 5-minute soak in water, B -- thiram-containing seed protectant dust, and C -- 5-minute soak in a 1000 ppm solution of phenacridane chloride.

FIGURE 3. Relative germination of Beefsteak tomato seeds at the end of 5 days' incubation. A -- untreated, B -- 5-minute soak in water, and C -- 5-minute soak in 1000 ppm phenacridane chloride.

Table 1. Effects of phenacridane chloride soak-treatment on percentage infestation and germination of tomato, pepper, and eggplant seeds.

Treatment	Tomato			Pepper			Eggplant		
	bacterial infestation	germination 5 days	germination 14 days	bacterial infestation	germination 6 days	germination 14 days	bacterial infestation	germinationd 7 days	germinationd 14 days
None	94(6)[a]	91	93	49(51)	89[c]	95	88(2)	90	91
Thiram fungicide	28	92	95						
Water	98(2)	94	96	60(40)	80	93	58(13)	93	94
PAC conc. (ppm)									
125	45	92	94				6(19)		
250	37	94	96	1(49)			1(3)		
500	3	95	96	1(9)	85	93	0	91	93
1000	1	94	95	0	84	94	0	88	91[e]
2000	0	94	96[b]	0	93	95	0	91	95[e]
4000	0	93	97[b]	0	88	95	0	89	92[e]

[a]Figures in parentheses show percentages of seeds infested with fungi.
[b]An average of 1% at 2000 ppm and 4% at 4000 ppm of seedlings developed necrotic areas on hypocotyl-radicle areas.
[c]Germination results of pepper seeds are from one experiment with 100 seeds.
[d]PAC-treated eggplant seeds were stored in sealed containers for 1 month before germination studies.
[e]An average of 0.5% of seedlings at the 1000, 2000, and 4000 ppm levels developed necrotic areas on hypocotyl-radicle areas.

Table 2. Efficacy of phenacridane chloride treatment in the reduction of bacterial infestation of freshly harvested, undried tomato seeds and stored tomato seeds[a].

Treatment	Percentage of seeds infested with bacteria	
	fresh	stored
Water	91[b]	92
PAC conc. (ppm)		
63	8	96
125	0	55
250	0	88
500	0	5
1000	0	0
2000	0	0

[a]Soak treatment.
[b]Data are averages obtained from an experiment with two replicates of 50 seeds per treatment.

Table 3. Germination percentages of phenacridane chloride treated tomato and eggplant seeds stored 1 month in open and sealed containers[a].

Treatment	Tomato				Eggplant			
	5 days		14 days		7 days		14 days	
	open	sealed	open	sealed	open	sealed	open	sealed
None	92	90	97	96	84	87	87	88
Thiram fungicide	92	94	98	96				
Water	92	90	98	96	84	93	86	93
PAC conc. (ppm)								
500	95	96	99	98	94	91	94	93
1000	93	95	96	96	92	84[b]	93	89
2000	94	94	97	99	80	88[b]	83	94
4000	95	93	97	94	87	84[b]	91	88

[a]Soak treatment.
[b]Under sealed storage an average of 1% of seedlings at the 1000, 2000, and 4000 ppm levels developed necrotic areas on hypocotyl-radicle areas.

Table 4. Antimicrobial activity of the phenacridane chloride retained by tomato seed surfaces following dip and soak treatment.

Treatment	Percentage infestation					
	Seeds aseptically planted		Seeds infested by handling		Seeds infested with a suspension of Xanthomonas vesicatoria	
None	90(10)[a]		100		100	
Thiram	30(3)		75		100	
	dip	soak	dip	soak	dip	soak
Water	100	98(2)	100	100	100	100
PAC conc. (ppm)						
500	17(7)	2(4)	---	4	---	4
1000	1(3)	0	0(4)	0	3(1)	0
2000	0	0	0	0	2	0
4000	0	0	0	0	0	0
8000	0	---	0	---	0	---

[a]Figures in parentheses are percentages of seeds infested with fungi.

Relative Disinfestant Activity of Phenacridane Chloride Applied to Freshly Harvested and Stored Seeds: Freshly harvested, undried tomato seeds required a concentration of only 125 ppm phenacridane chloride for complete disinfestation whereas stored, dry seeds required a concentration of 1000 ppm of the chemical (Table 2). Although the method of extraction of seeds in the laboratory was different from that used in commercial extraction, these results suggest that if commercial application of PAC were made after seeds were spin-dried but before they were air-dried disinfestation could be accomplished with far less chemical.

Resistance of Seeds Disinfested with Phenacridane Chloride to Artificial Inoculation: Tomato seeds treated with concentrations of 2000 and 4000 ppm PAC by either dip or soak application resisted contamination by intentional exposure to saprophytic bacteria present on the skin of fingers and palms (Table 4). In addition, when a drop of broth culture of Xanthomonas vesicatoria was used in an attempt to inoculate superficially 50 to 100 treated seeds, no infestation occurred on seeds that had been dipped or soaked 2 to 4 weeks earlier in 4000 ppm solutions of the chemical. The amount of phenacridane chloride that persisted on seeds dip-treated with 2000 ppm was not sufficient to prevent contamination completely and 2% of the seeds were infested with X. vesicatoria. Soak treatment, on the other hand, at 1000 and 2000 ppm resulted in resistance of seeds to contamination with this bacterial spot organism.

Depth of Phenacridane Chloride Penetration into Seeds: The only apparent difference between seeds treated by dip and soak methods was that the soaked seeds were stained more intensely than the dipped. To ascertain whether microscopic differences of greater magnitude existed between dip and soak-treated seeds, seeds were dipped or were soaked for 2.5, 5, 10, or 20 minutes in a 4000-ppm solution of phenacridane chloride or water and air-dried for 24 hours. Fifty-micron sections of comparable areas of the chemical-treated, water-treated, and untreated seeds were cut with a freezing microtome and mounted in glycerin.

Microscopic examination of the sections from 5-minute soak treatment revealed that the phenacridane chloride, indicated by the intense yellow stain, was limited mainly to the seed coat. The yellow stain of the compound was equally intense in the outer (hair-like extensions) and inner areas of the coat. No stain was apparent in the internal tissues. As the time of treatment was increased from an instant (dip) through 5 minutes, intensity of the yellow stain in the seed coat increased but penetration of the stain into deeper tissues did not occur. Seeds treated longer than 5 minutes did not show an increase in intensity of stain or a change in the position of the stain. Direct measurement of intensity of the yellow stain in the seed coat was not attempted. Photometric measurement (450 mμ) of the decrease in intensity of color was made after the seeds were removed from the PAC solutions. These measurements indicated the concentration of PAC remaining in solution after treatment which, of course, was inversely related to the amount of chemical held by the seeds. Expressed as $\frac{1000 \times \text{Optical Density}}{2}$ for the several periods of seed immersion the values are as follows: dip treatment, 100; 2.5-minute soak, 40; 5-minute soak, 35; 10-minute soak, 30; 20-minute soak, 30. Maximum uptake of PAC occurred after about 5 minutes' immersion. Estimation of intensity of stain in the 50-micron sections of representative seeds treated for the various intervals agreed with these findings. Neither the germination rate nor the total germination of seeds treated with PAC for the various intervals was affected detectably.

Leaching of PAC from treated seeds planted in soil should occur slowly since the chemical is distributed throughout the seed coat. An experiment to determine indirectly the rate that PAC was leached from seeds using tomato seeds dip-treated with 8000 ppm PAC showed that no gross reduction in intensity of the yellow color was apparent after 4 hours of continuous washing with tap water. Although after 24 hours' washing the yellow color of treated seeds was reduced to what appeared to be one-quarter the original intensity, these seeds did not give rise to any microbial growth when placed on nutrient agar.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

ALTERNARIA LEAF SPOT OF PIERIS JAPONICA[1]

George C. Hartmann[2]

Abstract

Alternaria tenuis[3] is reported as a pathogen of Pieris japonica apparently for the first time. On this host the fungus produces leaf spots up to 1 cm in size characterized by a reddish periphery and a tan center which at times is faintly zonate and on which develop pads of sporulating hyphae appearing as black specks to the naked eye.

INTRODUCTION

The ericaceous plant Pieris japonica, commonly called Andromeda, is an economically important ornamental that is widely grown in Rhode Island. While working as a nursery disease inspector during the summer of 1959, the author observed a number of Andromeda plants exhibiting leaf spots as large as 1 cm in diameter and characterized either by a reddish periphery and tan center with black specks or by alternating circles of brown and tan, that is, a target spot. Hyphae and spores of an Alternaria were observed in cross sections of the lesions. Although the Index of Plant Diseases in the United States[4] lists only Pestalotia sp. and Phyllosticta andromeda West. as the causal agents of leaf spots of P. japonica, the author consistently isolated a fungus referable to Alternaria tenuis[3] from leaf spots and stem lesions. Therefore an investigation of the pathogenicity of this fungus on P. japonica was undertaken.

METHODS AND MATERIALS

In the inoculation experiments seedlings of P. japonica, 3 to 5 inches tall, grown under greenhouse conditions were used as host plants. Inoculum was prepared from a 48 to 72 hour culture of a single spore isolate of the fungus grown on potato-dextrose agar. The epidermis on specific areas of leaves and stems was pierced with the point of a flamed needle, and an agar block, 2 mm x 4 mm, was applied to each wounded site. Each agar block was wrapped in moist cotton and the host plant covered with a bell jar which was removed after 24 hours. Inoculum and moist cotton were left in place for 24 hours longer and then removed. Sterile agar blocks were used on control plants. At 3- to 4-day intervals the width and length of both regular and irregular lesions occurring after inoculation were measured.

Thirty-five leaves on four plants were inoculated after injury, and on three plants 13 leaves were inoculated without prior injury. On five plants, 25 previously injured stem sites were inoculated and on three separate plants 11 stem areas were inoculated without previous injury. In all, 15 experimental plants were inoculated.

DISCUSSION OF RESULTS

A definite leaf spot was produced at each of the 35 leaf areas injured prior to inoculation, but no lesion was produced at the 13 uninjured inoculation sites. Lesions formed at 16 of the 25 stem areas injured before inoculation; however, no lesion ever resulted where the stem was not injured before inoculation.

Leaf spots, which were visible within 2 days after inoculation, were characterized by a reddish, circular or irregular periphery and a tan center sometimes faintly zonate. They ranged in size from 2 mm in diameter to 5 x 7 mm. A cluster of small spots coalesced to form a single spot. Leaf spots generally reached a maximum size within 5 days after inoculation. Each lesion extended from the upper epidermis through the mesophyll tissue to the lower epidermis. Ten days after inoculation black acervuli-like specks appeared on the tan center of the

[1]The author gratefully acknowledges the help and advice of Dr. Nestor E. Caroselli, Professor of Botany, University of Rhode Island.

[2]Assistant Professor of Biology, Rhode Island College.

[3]The author wishes to thank Dr. Emory G. Simmons of the Quartermaster Research and Engineering Center, Natick, Massachusetts, who identified the fungus. Dr. Simmons states that this isolate varies somewhat from typical A. tenuis but due to the lack of a satisfactory taxonomic treatment of this genus he feels that "A. tenuis group" is the best designation at the present time.

[4]United States Department of Agriculture. 1960. Index of Plant Diseases in the United States. Agriculture Handbook No. 165.

spot on the upper leaf surface and after 2 more days on the undersurface as well. Microscopic examination clearly revealed the specks to be pads of sporulating hyphae. Approximately 10% of the lesions exhibit a discontinuous chlorotic halo around the periphery within 1 month after inoculation. The spots developing from greenhouse inoculations were similar to those observed on nursery plants.

A. tenuis was consistently re-isolated from leaf lesions (although it could not be re-isolated from stem lesions) and found to be morphologically similar to the original isolate in all cases. Under the microscope the characteristic hyphae of A. tenuis were seen in the green leaf tissue around the leaf spot. They extended through the intercellular spaces of the spongy mesophyll and upward between the palisade cells; the vascular tissue was not affected. A few of the mesophyll cells seemed to contain a short haustorium-like structure.

The aforementioned observations indicate that A. tenuis is a weak pathogen of leaf tissue of P. japonica.

DEPARTMENT OF BIOLOGY, RHODE ISLAND COLLEGE

PRATYLENCHUS ZEAE FOUND ON CORN, MILO, AND THREE SUSPECTED NEW HOSTS IN CALIFORNIA

Sadek M. Ayoub[1]

Examination of a soil sample collected in the course of plant disease detection surveys conducted by the Bureau of Plant Pathology, California Department of Agriculture, and county departments of agriculture in 1961 revealed the presence of Pratylenchus zeae Graham, 1951 in a commercial field of milo (Sorghum vulgare) in Fresno County. This is the first report of an established P. zeae infestation in California.

Resampling of the original 9-acre field has shown the nematode to be generally present in the soil and in the milo roots. Surveys of milo and corn fields in the surrounding area revealed the presence of P. zeae in two additional milo fields and one corn (Zea mays) field within a 2-mile radius.

Root samples of Bermuda grass (Cynodon dactylon), puncture vine (Tribulus terrestris), and watergrass (Echinochloa crus-galli) were collected from the original infested milo field and carefully washed to remove adhering soil. By means of Young's jar incubation method (6), Pratylenchus zeae was found in association with the roots of each of these weeds, none of which has been previously reported as a host for this nematode. No greenhouse trials have been conducted to study the nematode-host relationship, but it is possible that these plants may serve as reservoirs of P. zeae between plantings of host crops.

Hosts of P. zeae published in the literature include: Digitaria sanguinalis, crabgrass (2); Glycine max, soybean (2); Nicotiana tabacum, tobacco (3); Prunus persica, peach (1); Saccharum officinarum, sugarcane (1); Secale cereale, rye (2); Setaria italica, millet (2); Sorghum halepense, Johnson grass (5); Sorghum sudanense, Sudan grass (2); Sorghum vulgare, milo (2); Sorghum vulgare, sorghum (2); and Zea mays, corn (2, 3, 4).

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BUREAU OF PLANT PATHOLOGY, CALIFORNIA DEPARTMENT OF AGRICULTURE, SACRAMENTO, CALIFORNIA

[1]Plant Nematologist, Bureau of Plant Pathology, California Department of Agriculture, Sacramento, California.

FIELD AND HOST STUDIES OF PARASITISM BY HELMINTHOSPORIUM SOROKINIANUM

Harvey W. Spurr, Jr. and Richard L. Kiesling[1]

Summary

Sixteen barley varieties were tested for resistance to Helminthosporium sorokinianum Sacc. ex Sorokin at three locations in Michigan. No resistance was found. The severity of infection in varieties varied between the nurseries. A rotation of barley after barley may explain the heavier infection at one nursery. Sporulation of H. sorokinianum on barley straw prior to harvest could explain an increase in air-borne conidia observed. Sporulation appeared on areas of barley straw exposed to direct sunlight. Beans, tomatoes and certain other dicotyledonous plants were parasitized by H. sorokinianum in greenhouse host range studies. H. sorokinianum was isolated from leaf lesions on field grown Michelite beans. The symptoms on bean leaves may serve to distinguish H. sorokinianum from H. victoriae Meehan & Murphy.

INTRODUCTION

Helminthosporium sorokinianum has been a problem since 1910 when it was shown to be a plant pathogen (5). The fungus causes seedling blight, root and culm rots, leaf spots and kernel and head blights on cereals. Reduction in yield as a result of infection is reported to be 12% per year in wheat (2). Some of the phases of the disease caused by this fungus can not be controlled. This study was conducted to examine further the host range and epidemiology of H. sorokinianum.

MATERIALS AND METHODS

Barley nurseries were established at East Lansing, Standish and Tuscola, Michigan, to determine the reaction of 16 barley varieties to H. sorokinianum. These varieties were CI 187, 198, 691, 711, 731, 739, 1245, 1367, 1517, 1907, 2276, 4578, 4979, 7269, 5105, 6969 and were selected because of their reported resistance to H. sorokinianum. The nursery locations represent three barley-growing areas in Michigan. Infection of culms and leaves was evaluated after the plants headed. Sporulation on the straw was studied at the East Lansing nursery.

Soil- and seed-borne inoculum of H. sorokinianum was considered to be the source of seedling blight, root and culm rot and leaf spot infections on barley. The source of inoculum causing kernel and head blights was unknown. A large barley field, including the East Lansing barley nursery, was selected for studying air-borne inoculum. Six vaseline-coated microscope slides were placed vertically 6 inches above the ground on stakes. The stakes were placed within a 2-acre area of the barley field with the slides facing the prevailing winds. The slides were replaced every 7 days. After the slides were removed from the field, a 1-inch square cover slip was placed on the vaseline in the center of each slide. The slides were read under low power of the microscope by passing across the cover slip three times at 5-mm intervals. The average number of conidia counted per slide per each group of six slides was recorded. Records were made from April 27 to August 26, 1957.

H. sorokinianum is known primarily as a cereal pathogen (4). An incident of H. sorokinianum seedling blight of wheat which followed Michelite beans in rotation lead to further study of the host range. Twenty-four plant species and varieties, including several from dicotyledonous genera, were inoculated in the greenhouse. The plants were grown in 4-inch pots in the greenhouse and were sprayed with a distilled water suspension of conidia when several leaves had developed. Isolate H. S. 101, from barley, single-spored for six generations, was used for all inoculations. The plants were placed in a moist chamber for 2 days at 72° F after being inoculated and then returned to the greenhouse bench. Nine days after the inoculation the plants were examined and isolations made from suspect lesions. The experiment was repeated several times and the following technique was used for the successful isolation of H. sorokinianum from lesions: a disk of tissue was removed from the edge of a lesion with a 3/8-inch cork borer, the disks were placed in a Gooch crucible and dipped in a 1:1:1 solution (95% ethanol : commercial sodium hypochlorite : distilled water) for 5 seconds, the disks were then placed on potato-dextrose agar acidified with five drops of lactic acid per 200 ml agar.

[1]Postdoctoral Fellow, Department of Plant Pathology, University of Wisconsin and Professor and Head, Department of Plant Pathology, North Dakota State University, respectively.

RESULTS

Basal culm rot caused by H. sorokinianum was present on all varieties at the three nurseries. Leaves were also infected although the damage was not extensive, and no sporulation was observed on the leaves during the growing season. Disease development was most severe at the Tuscola nursery. None of the varieties were resistant, and the severity of infection in varieties varied from nursery to nursery. Sporulation on the straw appeared to be directly related to the severity of infection. Extensive examination of the sporulation on barley straw indicated that it was located mainly on areas exposed to direct sunlight (Fig. 1).

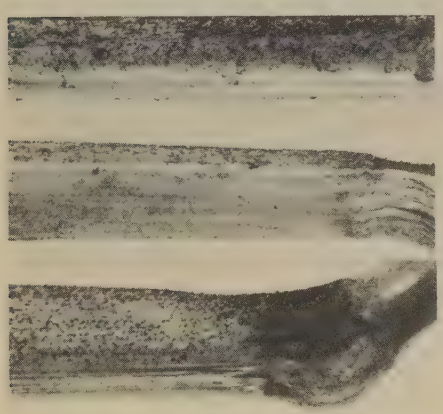

FIGURE 1. Sporulation on areas of barley straw exposed to direct sunlight. From top to bottom: sporulation of H. sorokinianum and Alternaria sp., slight sporulation of H. sorokinianum, heavy sporulation of H. sorokinianum.

In the study of air-borne inoculum, conidia of H. sorokinianum were not found on the slides until late in the growing season. The barley headed by June 20. Sporulation of H. sorokinianum was first observed on the barley straw on July 31. The first slides containing H. sorokinianum conidia were removed from the field on August 3 and averaged 4.5 conidia counted per slide. All slides thereafter until the experiment terminated averaged two conidia.

Several dicotyledonous plants were infected by H. sorokinianum under greenhouse conditions in the host range study (Table 1). When lesions appeared they were usually numerous and distributed over the entire leaf surface. On bean leaves the lesions were round, 1/2 to 1 mm in diameter, brown to black and visible to both leaf surfaces.

The avid parasitism of bean leaves by H. sorokinianum observed in the greenhouse prompted an investigation of a field of Michelite beans. The field was adjacent to and had the same crop rotation as the Tuscola nursery. Eight leaves were selected and iso-

Table 1. Plants inoculated with conidia of H. sorokinianum.

	Plant host	Trial III L[a]	R[b]
Bean:	BlueLake	+	+
	Fordhook No. 242	+	+
	Great Northern	+	+
	Idaho	+	+
	Michelite	+	+
	Pencil Pod Black Wax	+	+
Crucifers:	Chinese Mandarin	-	-
	Danish Baldhead	-	-
	Premium Flat Dutch	-	-
	Wisconsin Hollander	-	-
	Early Scarlet Radish	-	-
Corn:	Golden Cross Bantam	+	+
Cowpea:	Black	+	+
Cucurbits:	National Pickling	+	+
	Mammoth King Pumpkin	+	+
	Wild Cucumber	-	-
Horsebean:		+	+
Pea:	Alderman	+	+
Small grain:	Barley 934	+	+
	Barley 5105	+	+
	Winter Oats	-	-
Sunflower		+	+
Tomato:	Bonny Best	+	+
	Rutgers	+	+

[a] L (+) indicates lesion produced.

[b] R (+) indicates reisolation of H. sorokinianum from lesions.

lations were made from 58 suspect lesions using the above-mentioned technique. H. sorokinianum was isolated from four lesions (3.5%).

DISCUSSION AND CONCLUSIONS

The infection of the 16 barley varieties by H. sorokinianum as well as the infection of several dicotyledonous plants and field-grown Michelite beans emphasizes the host range of H. sorokinianum. The influence of crop rotation may explain the heavier infection observed at the Tuscola nursery. Broadfoot (1) observed that foot rot on wheat caused by H. sorokinianum was increased when wheat followed wheat, barley or western rye grass. The Tuscola nursery, unlike the other nurseries, had barley after barley. Thus, the proper rotation of crops under Michigan conditions might reduce H. sorokinianum infections by reducing the inoculum available since sporulation on barley straw appeared to be directly related to the severity of infection. However, crops proposed for such rotations should be evaluated for resistance to local isolates of H. sorokinianum before being employed in short rotations.

The importance of air-borne inoculum of H. sorokinianum in head blight development remains to be clearly established. Although an increase in air-borne inoculum was observed, this increase was slight and occurred during the time the kernels were almost mature and shortly before the harvest. An increase in air-borne conidia was observed by Machacek and Greaney (3) in Manitoba about the time the kernels were maturing.

Winstead and Hebert (6) reported natural and artificial infection of beans with Helminthosporium victoriae. They reported that the leaf lesions were primarily on the veins of the lower surfaces and were often brown to black, narrow streaks 1 to 5 mm in length. In contrast, H. sorokinianum infection of bean leaves had no affinity for veins, no apparent preference for either leaf surface and produced only round lesions. These differences may be a way to distinguish these species in the future.

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DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, MICHIGAN STATE UNIVERSITY, EAST LANSING, MICHIGAN

POWDERY MILDEW ON SAFFLOWER[1]

D. E. Zimmer[2]

Powdery mildew caused by a species of Erysiphe was discovered on seedlings and young plants of Nebraska 1-1-5, Nebraska 6, Nebraska 10, Nebraska 977-15-1, Pacific 2, and Utah 1421 safflower grown in greenhouses at Logan, Utah, during the summer of 1961. This disease has not previously been reported on safflower in the United States. Ashri[3] reported powdery mildew on field-grown safflower in Israel but did not identify the causal agent.

White mycelial tufts were observed on the cotyledons and seedling leaves soon after emergence. Tufts of mycelium were abundant on the base of stems and leaf petioles. Heavily infected leaves became chlorotic and died prematurely, suggesting the potential severity of this disease under ideal field conditions. Perithecia were observed on infected leaves, petioles, and stems prior to death of the leaves or maturity of the plant.

Diagrams of the causal agent appear in Figure 1. The range and mean number of asci per perithecium, perithecial dimensions, and ascospore dimensions are recorded in Table 1. Microscopic studies of the perithecia and asci placed the causal organism in the genus Erysiphe, but positive species identification is not complete.

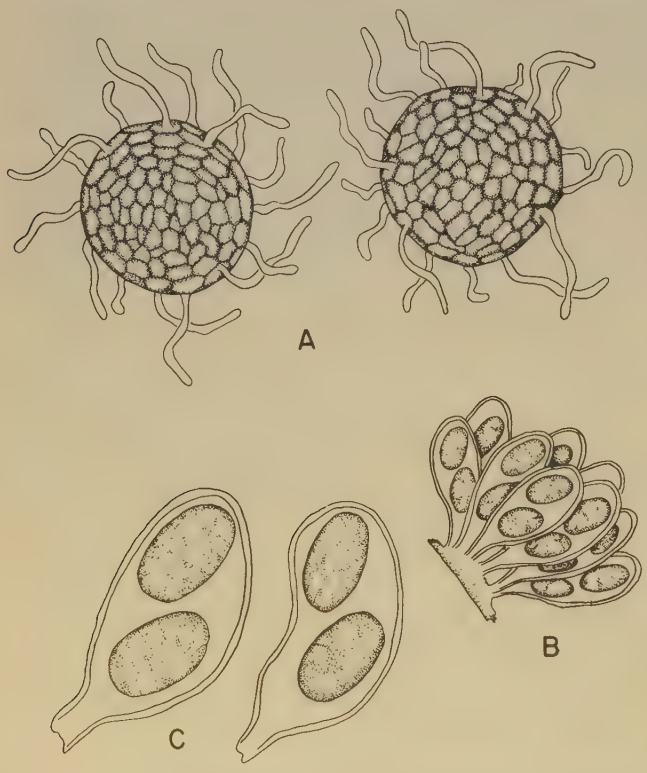

FIGURE 1. Diagrams of the sexual fruiting structures of safflower powdery mildew. A -- Mature perithecia with appendages; approx. X 187. B -- Cluster of asci from one perithecium; approx. X 225. C -- Asci with two ascospores, the normal number; approx. X 525.

Table 1. Numerical range and mean of asci per perithecium and perithecial dimensions, and ascosporic dimensions of safflower powdery mildew[a].

Statistic	Asci per perithecium (number)	Ascospores: length	Ascospores: width	Perithecial diameter
Range	5-12	17.5-35.0	13.4-20.0	94-152
Mean	9	26.8	16.7	124

[a]Based on 50 individual measurements.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE AND UTAH AGRICULTURAL EXPERIMENT STATION, LOGAN

[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Utah Agricultural Experiment Station, Logan, Utah. Approved as Journal paper No. 213. Utah Agricultural Experiment Station.

[2]Research Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

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OBSERVATIONS ON DECLINING SOUR ORANGE SEEDLING TREES IN SPAIN[1]

I. Reichert and O. Ginsburg[2]

Summary

Occasional sour orange seedling trees growing commercially in extensive orchards in Spain were found to be affected by general decline: mild and strong defoliation and desiccation of branches.

Bark sampling at various heights of the declining trees revealed the presence of pits and of ridges on the bark's cambial face.

The pinhole-like pits resulted from hyperplasia and sclerification of the rays. In diseased trees, there was a narrow ring of functional phloem (150-300μ compared with 450-500μ in healthy trees). In addition to these symptoms, a band of necrotic cells was observed in the functional phloem in some trees. The anatomical nature of the ridges was not investigated.

It was decided to name the Spanish sour orange seedling decline, "Sour orange seedling tree decline."

In October 1957 the senior author visited four sour orange seedling tree plantations in the province of Seville, Spain, where an estimated 400,000 sour orange trees are grown. Most of the trees are healthy, often attaining an age of 100 years or more, and yield approximately 300 kg of fruit per tree.

Only a small number of trees showed signs of mild or strong decline: defoliation and desiccation of branches (Fig. 1).

A preliminary report on this decline was made elsewhere (3, 4). This paper is the report of a more complete study of the results obtained.

MATERIALS AND METHODS

Of the four orchards visited, three were on heavy soil and one on lighter soil. In the first orchard, five declining and one healthy tree were examined; in the second, three declining and one healthy; in the third, one declining and one healthy tree; and in the fourth, two healthy and two declining.

Bark samples were taken at various heights from declining and healthy trees, macroscopically examined on the spot, and microscopically examined a year later at Rehovot. Cross-sections, 16-18μ thick, were made by means of a sliding microtome and then progressively stained with dilute Heidenhain's haematoxylin and lacmoid according to Schneider's methods (13).

OBSERVATIONS

Macroscopic: When bark was stripped from various heights of the stem, patches of small, round, pinhole-like pits and longish ridges could be seen on the undersurface of the bark (Fig. 2). On the corresponding positions on the wood there were tiny cylindrical woody pin-like outgrowths which fitted into the pits in the bark, and bigger elongated pits which corresponded to the longish ridges. The ridges appeared in all bark samples from the declining trees; whereas, in the bark samples from the healthy trees they were rare or absent. The ridging on the bark and corresponding pitting in the wood resembled that which appears on sweet lime and other rootstocks grafted to sweet oranges and affected by xyloporosis (5, 7).

Microscopic: The pinhole-like pits on the undersurface of the bark and the corresponding pin-like protruding outgrowths on the wood may be considered to be hyperplastic woody rays (1, 2, 6, 7, 8, 9, 11, 12). At the point of bark slippage from the wood, the lignified phloem rays remain attached to the wood, thus forming pits and pegs (Fig. 5). The functional phloem was less

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[2]Professor of Plant Pathology and Assistant Plant Pathologist, respectively, of the National and University Institute of Agriculture, Rehovot, Israel.

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than in heatlhy trees (Fig. 4). In sections from one sample we found a width of only 150μ, and in two other cases widths of 250μ and 350μ, respectively. The radial width of the functional phloem in bark sections from healthy trees was found to be between 450-500μ (Fig. 3), resembling that found by Schneider (10) in sour orange seedling trees in California.

In two of the declining trees, a band of necrotic sieve tubes arranged in a concentric pattern was observed in the functional phloem. This band consisted of from four to five rows of necrotic cells (Fig. 6).

DISCUSSION

The anatomical symptoms described above show some similarity to occasional atypical samples from healthy sour orange seedling trees as described by Schneider (10) in California. This author found phloem containing bands of necrotic sieve tubes, concentric with the functional phloem and a degeneration of unusually large numbers of old sieve tubes on the outer portion of the functional phloem.

Certain of the pathological features of the Spanish sour orange seedling disorder also occur in the sour orange rootstocks of trees budded to citrus and affected by various diseases and disorders (8, 9, 11, 12). One occasional symptom of these bud union diseases is that of hyperplasia and lignification of the rays which are expressed macroscopically as pin-like projections and pinhole-like pits. Such hyperplastic woody rays are commonly found in citrus trees devitalized by diseases (1, 2, 7, 8, 9, 11, 12), and the ring of functional phloem is narrow in them.

Because of the hyperplastic phloem rays and the wide bands of degenerated but not crushed phloem, the Spanish sour orange seedling decline had been identified, by the first author, with chronic decline, a bud union disorder (3). A reconsideration of the problem shows that it would be better to keep these two disorders separate, because the suscepts are different and one disease is a bud union disorder and the other is not. It would, therefore, be better to call the Spanish decline of sour orange seedling, "Sour orange seedling tree decline."

FIGURE 1. Sour orange seedling tree from Seville province, Spain, affected by mild decline only.

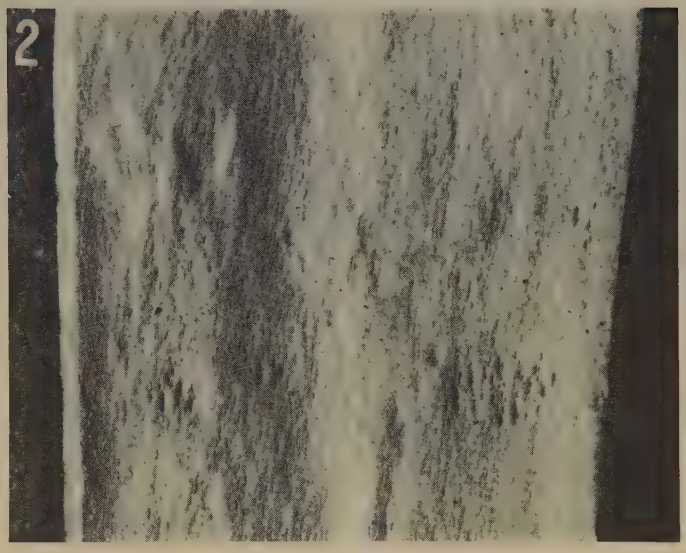
FIGURE 2. Bark of sour orange seedling trees in Spain, affected by sour orange seedling tree decline showing inverse pitting on the inner surface. Longish ridges may also be seen.

FIGURE 3. Cross-sections of phloem of a trunk of a healthy sour orange seedling tree in Spain showing a normal functional phloem. Approx. X 90.

FIGURE 4. Cross- section of phloem of the trunk of a declining sour orange seedling tree in Spain showing a narrow band of functional phloem and a broad band of degenerating phloem. One of the rays is hyperplastic ending in a small cavity formed by pulling out part of the ray upon removal of the bark. Approx. X 90.

FIGURE 5. Cross-section of phloem of the trunk of a declining sour orange seedling tree trunk showing a broad hyperplastic ray and a deep cavity. Besides, necrotic sieve tubes appear as a narrow band in the functional phloem. Approx. X 90.

FIGURE 6. Cross-section of phloem of the trunk of the declining seedling tree in Figure 5, showing a band of necrotic sieve tubes concentric with the functional phloem. Approx. X 60.



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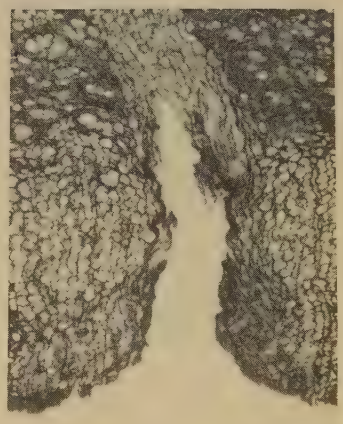

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DIVISION OF PLANT PATHOLOGY OF THE NATIONAL AND UNIVERSITY INSTITUTE OF AGRICULTURE, REHOVOT, ISRAEL

HOST RANGE AND INSECT TRANSMISSION OF THE HOJA BLANCA DISEASE OF RICE[1]

Guillermo E. Galvez, H. David Thurston, and Peter R. Jennings[2]

Summary

The results of these studies indicate that Sogata orizicola is the vector transmitting the hoja blanca virus to rice, that both S. orizicola and S. cubana can pass the virus from rice to Echinochloa colonum, the most common weed in Colombian rice fields, and that S. cubana is apparently the principal vector able to transmit the virus from grass to grass. In addition to Echinochloa, five other grass species have been infected with the hoja blanca virus from rice including wheat, barley and oats. Neither insect species has passed the virus from grass to rice in repeated tests.

Hoja blanca, a virus disease of rice, which apparently has been endemic in Colombia since at least 1935 (7, 8), has received considerable attention since 1956 because of the alarming losses it has caused in several Latin American countries. The disease is notably sporadic in occurrence, not only from season to season and zone to zone, but also among simultaneous plantings of the same variety within the same general area.

Improved long-grain varieties, preferred in many Central and South American countries, are all highly susceptible to the disease. Neither insecticide applications to control the vector nor changes in cultural practices (fertility levels, stand density, and irrigation) have shown value as control measures. Plant breeding, primarily with japonica type rices as sources of resistance in a backcross program, appears to be the only feasible means of control. Preliminary data indicate that resistance is conditioned by a single dominant gene but that minor gene action may be operative in certain variety combinations (2).

The hoja blanca virus is not transmitted by soil, seed, or mechanical means (4). A planthopper, Sogata orizicola Muir, has been shown to be the vector involved in its transmission to rice (1, 4). Several members of the Gramineae, including the cultivated crops wheat (Fig. 1), barley, oats, and rye, have been infected with the virus in cage tests (6, 10). A recent natural infection in wheat and oats seeded adjacent to rice has been reported (9). Symptoms on these crops and on grass weeds are similar to those on rice.

A preliminary report has shown that Sogata cubana Crawf. transmits the virus to Echinochloa colonum, a common rice weed frequently observed to have symptoms of the disease in nature (5).

This paper summarizes the data realized to date regarding the interrelationships of the two vector species in the transmission of the virus to rice and other hosts.

MATERIALS AND METHODS

All of the transmission studies reported were conducted in a screened greenhouse at the Ministry of Agriculture Experiment Station in Palmira, Colombia. The fact that no disease has ever been observed on uninoculated plants in the greenhouse indicates that contamination or uncontrolled infection did not occur in these studies. Uncontrolled temperatures within the greenhouse ranged from 16.5° to 44° C, with an average temperature of about 28°.

Two sizes of transparent plastic cages were used, one for entire plants and one for single leaves (Fig. 2). Insect transfers were made with glass aspirators. In transmission tests, unless otherwise indicated, insects were allowed to feed 3 to 4 days on diseased caged plants and were then transferred to healthy plants where they remained until death. Checks, used throughout these tests and consisting of 1 to 10 plants caged without insects in each of 10 cages, at no time showed symptoms of hoja blanca. Bluebonnet 50, a highly susceptible rice variety, was used in all tests.

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[1]Paper No. 140 of the Agricultural Journal Series of The Rockefeller Foundation.

[2]Plant Pathologist of the Colombian Ministry of Agriculture, Palmira, and Plant Pathologist and Associate Geneticist, respectively, of the Colombian Agricultural Program of The Rockefeller Foundation. The authors are indebted to Dr. Myron K. Brakke, University of Nebraska, for his help with the manuscript and with the interpretation of results, and to Ing. Agr. Enrique Torres and Sta. Alicia Pineda for technical assistance.

FIGURE 1. Symptoms of hoja blanca on wheat. Left, healthy spike; right, diseased.

FIGURE 2. Types of cages used for transmission tests with insects.

RESULTS

Transmission of Hoja Blanca Virus by Sogata orizicola:

1. Rice to rice. To date, 1035 adults of S. orizicola collected in rice fields have been tested individually for ability to transmit the virus from rice to rice after feeding on diseased plants. Of this number, 46 males and 56 females, or 9.9% of the insects tested, have transmitted hoja blanca. Thus, the great majority of the wild-type population appears to be unable to pass the virus even when previously exposed to diseased plants.

Several proven female transmitters were mated with either proven male vectors or with non-vectors and 355 nymphs from the resulting progenies were tested individually using the small plastic cages. The nymphs fed for 1 day on diseased rice and were transferred to healthy rice and left until death. Of the nymphs tested, 73 (23%) transmitted the disease. In 33 instances initial disease symptoms appeared before the nymphal stage was completed.

In these tests symptoms generally appeared 11 to 12 days following placement of the insect on the healthy plant, although symptoms have been noted as early as 5 days when both adults and nymphs were used. Symptoms initially appeared on the first or second leaf superior to that fed upon by the insect, but never on the inoculated or inferior leaves, an indication of an unidirectional movement of the virus within the plant.

2. Rice to other hosts. Table 1 gives the results of transmission tests with S. orizicola from rice to other grasses. In all cases the insects were fed for 2 days on diseased rice and were then transferred to the healthy grass growing in cages. All plants tested were grown from seed or vegetative plant parts. None of the check plants held in the greenhouse for an extended period developed symptoms. Most insects died after 2 to 3 days on the various species tested, although in a few cases involving Echinochloa colonum they lived as long as 10 days. Six of the species tested developed hoja blanca (Table 1).

3. E. colonum to E. colonum. One hundred S. orizicola fed on diseased E. colonum for 3 to 4 days and were then transferred in groups of 10 to 10 cages of healthy plants grown from seed. No diseased plants resulted.

4. E. colonum to rice. A total of 840 S. orizicola were fed on diseased E. colonum and then transferred in groups of 10 to 84 cages of healthy rice. No successful transmissions resulted.

Transmission of Hoja Blanca Virus by Sogata cubana:

1. Rice to rice. A total of 950 S. cubana were fed for 2 days on diseased rice and then transferred in groups of 10 and 20 to healthy rice in 80 cages. This vector does not survive well on rice. In one group of 2000 insects fed on diseased rice, only 250 lived to be placed on disease-free plants. No hoja blanca symptoms were observed on the caged plants.

2. Rice to E. colonum. The results of transmission tests from rice to E. colonum by S. cubana are given in Table 2. The insects fed on diseased rice plants for 3 days and were then transferred to E. colonum.

3. E. colonum to E. colonum. A total of 230 S. cubana were fed on diseased E. colonum for 2 days and were then transferred in groups of 10 to 23 cages of healthy plants grown from seed. Nine cages contained diseased plants at the end of the test. Symptoms were observed approximately 25 days following placement of the insect on the grass. Of 46 insects tested individually, five succeeded in transmitting the virus. Whereas S. cubana will not survive on rice, it lives exceptionally well on E. colonum.

4. E. colonum to rice. After feeding on diseased E. colonum, 300 S. cubana were transferred in groups of 10 and 20 to 25 cages of rice. No transmissions resulted in these tests or with 73 insects tested individually. The vector lived only about 2 days on the rice following transfer from the grass.

5. Rice to other grasses. The results of transmission from rice to other grasses by S. cubana are given in Table 3. Insects were fed for 2 days before being transferred to the tested grasses. This vector lived for very short periods on rice, sugarcane, barley, corn and sorghum and only slightly longer on feathergrass and pangola grass.

Field Collections of the Two Insect Vectors:

Weekly collections of the two species of Sogata were made for a period of 30 weeks in separated fields of rice and Echinochloa to determine the population frequency of the two vectors on the two plant species in nature. Only adult insects were counted since nymphs are difficult to separate into species. The counts presented in Table 4 show that S. orizicola constituted 85.5% of the insects collected in rice and only 3.06% of those found in Echinochloa, while the converse relation was true for S. cubana.

DISCUSSION

The wide range of temperatures recorded while the experimental work was in progress undoubtedly exercised an unfavorable influence on the number of proved transmissions of the virus and on the ability of the two vector species to survive on the various hosts tested. Rice appears to be the favored host of S. orizicola, since this leafhopper did not complete its life cycle on other plants in the greenhouse and in the field collections it was found predominantly on rice. S. cubana prefers and thrives on Echinochloa, judging from the facts that it did not survive on rice in the greenhouse and was found mainly on Echinochloa in the field.

The data indicate that S. orizicola is the vector transmitting the virus to rice, that both insect species can pass the virus from rice to Echinochloa, the most common weed in Colombian rice fields, and that S. cubana is apparently the principal vector able to transmit the virus from grass to grass. Neither insect species has passed the virus from grass to rice in repeated tests. This suggests that grass in rice fields acts as a biological trap for the virus and that weeds do not serve as sources of infection for rice. In this connection it is interesting to note that frequent observations have been made of rice fields completely or nearly free of hoja blanca while grass in the same fields was heavily diseased. Insect collections from these fields yield S. cubana predominantly. On the other hand, it is necessary to consider the possibility that in negative transmissions from Echinochloa to Echinochloa and rice with S. orizicola, and from rice and Echinochloa to rice with S. cubana, the insects did not live well on the tested plants and may have died before they had the chance to become viruliferous.

The experimental data and field observations suggest that seedings of rice and wheat in the same zone, which is a definite practicality in certain areas of Colombia, may result in severe losses in the wheat by transmission of the virus from rice by S. orizicola.

The grass hosts do not appear to play an important role in the epidemiology of hoja blanca; however, conditions necessary for severe epiphytotics are rather common in Colombia, since the farmers grow rice the year around and use varieties highly susceptible to the virus.

Table 1. Results of inoculations of hoja blanca virus from rice to other grasses by S. orizicola.

Grass tested	Insects per cage	Total number of: insects	cages	plants	Cages with plants showing symptoms	Plants caged without insects[b]
Jungle rice, Echinochloa colonum	10, 20, 50	630[a]	43	43	3	40
Feathergrass, Leptochloa filiformis	10, 20	310	21	21	4	20
African grass, Digitaria horizontalis	10, 20	500	30	30	1	30
Sugarcane, Saccharum officinarum	20, 30	700	30	30	0	10
Pangola grass, Digitaria decumbens	10, 20, 25	480	25	75	0	100
Barley, Hordeum vulgare, variety Funza	10, 20, 30, 40	920	54	275	1	100
Wheat, Triticum vulgare, variety Bonza	10, 20, 30, 40	800	35	175	3	100
Corn, Zea mays	10, 30	420	28	140	0	100
Oats, Avena sativa, variety CI 6969	10, 20, 30	680	41	123	2	100
Sorghum, Sorghum vulgare	10, 20	390	30	150	0	100
Rye, Secale cereale	10, 50	60	2	2	0	0

[a]Insects were collected in a rice field heavily infected with the virus.
[b]None of these plants showed symptoms of hoja blanca virus.

Table 2. Results of inoculations of hoja blanca virus from rice to E. colonum by S. cubana.

Insects per cage	Total number of: insects[a]	cages	Cages with plants showing symptoms	Plants caged without insects[b]
20	260	13	7	100
10	220	22	6	100
5	25	5	0	0
2	94	47	12	30
1	82	82	12	50

[a]Most of the insects were collected in a rice field infected with hoja blanca. A few insects were from a colony obtained under controlled conditions in the greenhouse.
[b]None of the plants showed symptoms of hoja blanca virus.

Table 3. Results of inoculations of hoja blanca virus from rice to other grasses by S. cubana.

Grass tested	Insects per cage	Total number of: insects	cages	plants	Cages with plants showing symptoms	Plants caged without insects[b]
Feathergrass	10, 20, 50	690[a]	20	20	2	10
African grass	20	500	25	25	0	10
Sugarcane	10, 20, 40	360	24	24	0	10
Pangola grass	10, 20, 40	650	30	90	0	50
Barley, variety Funza	10, 20, 40	970	37	185	0	100
Corn	10, 20, 30, 40	680	28	140	0	100
Oats, variety CI 6969	10, 20, 40	750	35	105	0	100
Sorghum	20, 40	520	16	80	0	50
Wheat, variety Bonza	10, 20, 40	700	30	150	0	100

[a]Insects were collected in a rice field heavily infected with the virus.
[b]None of the plants showed symptoms of the disease.

Table 4. Frequency of Sogata species in rice and Echinochloa in field collections.

	Rice[a]		Echinochloa[b]	
	S. orizicola	S. cubana	S. orizicola	S. cubana
No.	16,336	2,769	645	20,368
%	85.50	14.50	3.06	96.94

[a]Between 60 and 70% of the rice plants showed symptoms of hoja blanca.
[b]Between 70 and 80% of Echinochloa plants showed symptoms of hoja blanca.

A large supply of inoculum as well as suitable hosts and large numbers of vectors will be potentially present at the same time, providing optimum conditions for epiphytotics.

About 9.9% of the native population of S. orizicola was able to transmit the hoja blanca virus. This percentage was greatly increased when insects from active females were used. Storey (11), working with Cicadulina mbila Naudé, vector of the maize streak virus, and Black (3), with the potato yellow-dwarf virus vector, were able to develop active and inactive races of the insects by controlled crossing. Consequently, the increased transmission by insects from active females strongly suggests that a similar specificity in vector ability, genetically controlled, occurs in S. orizicola and perhaps also in S. cubana. Studies to clarify these observations will not only aid an understanding of such specificity but will also provide more information about the genetic combinations involved.

The vector-virus relationships have not been established since most of the insects used throughout this research were from the field. It has been observed, however, that 1 day is sufficient for an insect fed on an infected plant to become viruliferous. The minimum incubation period observed in rice plants was 5 days. Although no definitive data on incubation period in S. orizicola have been obtained, the fact that most plants developed symptoms 11 to 12 days after the beginning of inoculation feeding, or 12 to 14 days after the start of the acquisition feeding, points to an incubation period in the leafhopper of 7 to 9 days. In addition, short incubation periods were observed when some nymphs from active females were used. This suggests that the incubation period of the virus in the plant occurred without an incubation period in the insect because of transmission through the eggs. Furthermore, the insects were able to transmit the virus both in nymphal stage and as male and female adults, and they kept such ability throughout life.

If the suggestions obtained from transmission studies, including host range, are considered, the importance of undertaking further research to determine vector-virus relationships for the hoja blanca virus is clear. This information would aid a better understanding of the behavior of plant viruses transmitted by leafhoppers.

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THE COLOMBIAN MINISTRY OF AGRICULTURE AND THE COLOMBIAN AGRICULTURAL PROGRAM OF THE ROCKEFELLER FOUNDATION

RATOON STUNTING DISEASE OF SUGARCANE IN COLOMBIA[1]

Guillermo E. Galvez and H. David Thurston[2]

Abstract

A deterioration of the commercial varieties of sugarcane grown in Colombia has been observed for several years. Field tests have shown yield reductions due to the disease of about 65 to 70%. The causal agent is easily transmitted from diseased to healthy plant material by mechanical means. The disease may be controlled by hot-water treatment of seed pieces. These facts suggest that the cause of the disease is a virus whose symptomatology coincides with that described for ratoon stunting disease.

Ratoon stunting disease of sugarcane, a virus disease, has caused more serious losses to the sugar industry throughout the world than any other disease during the last 16 years. Bell (1) reported the disease for the first time in 1932; however, it became important only in 1945 when it was responsible for the deterioration of the promising variety Q. 28 in Queensland (3). The diagnosis of the disease has been based on the symptoms found in the nodal vascular tissue in mature and immature shoots, and on the reduction of the yield from plants grown from diseased material, especially from the ratoons (2, 3, 4, 5).

King (6) in 1954 reported symptoms similar to those of the ratoon stunting disease in Colombia. Ramos-Núñez (personal communication) in 1959 found symptoms in Colombia similar to those seen in infected sugarcane crops in the United States, Mexico, and Cuba. Recently studies have been undertaken to determine the cause of deterioration of the varieties grown in Colombia during the past several years. This paper presents the results obtained to date.

Description of the Disease: The external symptoms of the disease are stunting, reduced vigor of plants, and general chlorosis of the leaves. The internal symptoms consist of an orange to reddish discoloration of the vascular tissue at the base of the nodes of mature stalks. The number of vascular bundles affected varies within wide limits. A general, light pink discoloration is also present in the nodes of very young shoots.

MATERIALS AND METHODS

The experiments were carried out at the Ministry of Agriculture Experiment Station in Palmira, Colombia with the sugarcane variety EPC-33833. In the transmission tests the varieties Azul Casa Grande and F-31962 were also used.

For a yield test, apparently diseased plant material was selected and divided into two groups, one of which was treated in hot water at 51° C for 1 1/2 hours. In addition, the seed pieces were treated with a mercurial fungicide immediately before planting. A "split plot" experimental design was used, with irrigation as main plots and hot-water treatment as subplots. Six rows, 15.0 m long and 1.50 m apart, were planted per subplot with six replications of each treatment. The results were determined by weighing the harvest of sugarcane from the four central rows of each subplot 12 months after planting. Excessive rainfall during the experiment did not permit the application of water as planned, and therefore the main plots were not considered in the analysis of the experiment.

The mechanical transmission tests were made with apparently healthy material treated in hot water at 51° C for 1 1/2 hours. Juice from apparently diseased stalks was extracted in a small mill. The seed pieces were inoculated by several cuttings with a knife previously dipped in fresh extract of diseased stalks. The seed pieces were treated with a mercurial fungicide and planted. Plant material from the healthy source was planted without inoculation as a control. In addition, seed pieces from the stalks used as inoculum source were planted

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[2] Plant Pathologists of the Colombian Ministry of Agriculture (D. I. A.), Palmira, Colombia and the Colombian Agricultural Program of The Rockefeller Foundation, respectively. The authors are indebted to Dr. Myron K. Brakke, University of Nebraska, for his help with the manuscript, and to Ing. Agr. Enrique Torres T. and Sta. Alicia Pineda for technical assistance.

either with heat treatment or without as an inoculum control. At least 20 seed pieces were used per treatment, and were planted in the field in rows 10 m long and 1.50 m apart. The results were determined as the percentage of infected stalks 6 months after the planting date, and as the percentage and weight obtained from the first ratoon 6 1/2 months after the first cutting.

RESULTS

The results of the yield test from the plant crop are presented in Table 1. The reaction of the variety to hot-water treatment is highly significant and indicates that the apparently diseased stalks used as seed pieces were actually diseased. Losses due to the disease were at least 65 to 70% in the variety EPC-33833. Since no water was applied to the plots that were to be irrigated, these could be considered as six more unirrigated replications.

The results of the mechanical transmission tests (Table 2) indicate that the causal agent was easily passed from diseased to healthy plants by knife cutting, the most common method of harvesting sugarcane in Colombia. The data also show that the varieties reacted differently to the hot-water treatment, that is, Azul Casa Grande did not respond to treatment, and that the controls for inoculum and inoculation method worked satisfactorily.

Table 1. Yields obtained from apparently diseased plant material treated in hot water at 51° C for 1 1/2 hours and untreated.

Replication	Yield, in kg per plot (90 m^2)	
	untreated	treated
1	324.8	1,326.0
2	399.6	1,504.0
3	794.8	1,534.0
4	766.2	1,771.0
5	413.0	1,557.0
6	433.6	1,231.0
7	375.0	1,286.0
8	399.4	1,750.0
9	803.2	1,766.0
10	448.6	1,551.0
11	329.2	1,605.0
12	110.0	1,201.0
Total	5,597.4	18,082.0
Mean	466.4	1,506.8
LSD at 5%	113.04	
1%	159.52	

Table 2. Percentage of diseased stalks and weight for healthy, hot-water treated plants uninoculated and mechanically inoculated with juice from apparently diseased stalks.

Variety	Inoculated: plant crop: % diseased stalks	Inoculated: 1st ratoon: % diseased stalks	Inoculated: 1st ratoon: weight (kg)	Uninoculated: plant crop: % diseased stalks	Uninoculated: 1st ratoon: % diseased stalks	Uninoculated: 1st ratoon: weight (kg)
EPC-33833	92.0[a]	56.0	61.0	0.0	4.0	224.5
F-31962	93.3	84.0	32.0	1.1	0.0	105.0
Azul Casa Grande	87.7	66.0	126.5	82.2	70.0	101.0
	Not heat-treated			Heat-treated		
EPC-33833[b]	83.6	82.0	55.5	1.5	2.0	180.5

[a] Based on at least 20 plants.

[b] Inoculum controls: uninoculated plants grown from stalks used as inoculum source.

DISCUSSION

These data show that an easily transmissible disease of sugarcane is present in Colombia. Since the losses are relatively high, it may be the primary factor responsible for the rapid deterioration of the varieties grown in the major sugar-producing area, the Cauca Valley.

The transmission of the causal agent by mechanical means from diseased to healthy plant material as well as its control by heat applied for long periods at high temperatures suggest that this agent is a virus.

The symptomatology observed -- chlorosis of the leaves, discoloration of the nodal vascular tissue, and especially the striking difference in yield found when seed pieces were heat-treated -- coincides with that described for "ratoon stunting disease." Since identification of this virus depends on symptomatology alone, it could be assumed that the ratoon stunt virus is responsible for the stunting disease of sugarcane in Colombia. Similar symptoms may be caused by other conditions, however, and it cannot be definitely concluded that the disease in Colombia is the same as in other countries. Improved methods of identification are needed, since other viruses may be present not only in Colombia but elsewhere under the name of ratoon stunting disease of sugarcane.

Although control of the virus does not seem to be difficult, the temperature-time factor must be determined for each of the commercial varieties.

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THE COLOMBIAN MINISTRY OF AGRICULTURE AND THE COLOMBIAN AGRICULTURAL PROGRAM OF THE ROCKEFELLER FOUNDATION

EPIDEMICS OF PINE NEEDLE RUST IN ARKANSAS[1]

Charles L. Wilson[2]

In 1960 and 1961 there were epidemics of pine needle rust (Coleosporium spp.) in several young loblolly (Pinus taeda) plantations in southern Arkansas. Although needle rusts generally do not cause extensive damage, in some instances whole plantations were affected and trees were killed, apparently from defoliation by needle rust infections.

The development of needle rust was followed in one of the affected plantations during 1960. This was a 20-acre, 3-year-old loblolly plantation near Fordyce, Arkansas. It is on a bottomland site that was cultivated previously. There was an abundance of ironweed (Vernonia sp.) throughout the planting.

The needle rust in this planting was identified as Coleosporium vernoniae Berk. & Curt. Ironweed (Vernonia sp.) was the only possible alternate host nearby on which the uredial and telial stage was found. Ironweed was predominant throughout the planting and numerous uredia were present on the foliage examined. The aeciospores on the pine needles and the uredio- and teliospores on Vernonia corresponded to Arthur's description[3] of Coleosporium vernoniae.

FIGURE 1. Numerous aecia of Coleosporium vernoniae on loblolly needles. The aecial wall has not ruptured.

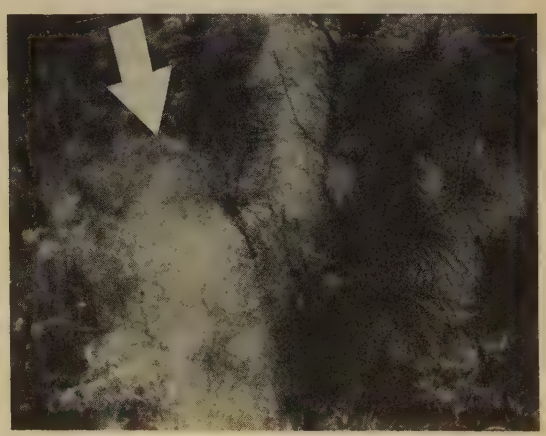

FIGURE 2. Cloud of aeciospores of Coleosporium vernoniae liberated from aecia (arrow). Tree was shaken prior to taking the photograph.

The aecial peridia were fully expanded on the needles, but had not ruptured on April 21 (Fig. 1). When observed April 26, the aecial peridia had ruptured and aeciospores were being liberated (Fig. 2). The uredial stage was collected May 24 on nearby ironweed plants (Vernonia sp.). No teliospores were found in the pustules at this time. Teliospores were found on material collected June 1.

All the plantations observed that had extensive damage from pine needle rust were on bottomland sites that had been cultivated previously. An abundance of composites was present within the severely affected plantings. It may be possible to control this disease in a limited area by eradicating the alternate host before the teliospores are produced.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF ARKANSAS, FAYETTEVILLE

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[1]Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

[2]Assistant Professor, University of Arkansas, Fayetteville.

The author is indebted to Dr. G. E. Templeton for the identification of C. vernoniae. Mr. Robert Clark, Chief Forester, Fordyce Lumber Company, called this problem to our attention.

[3]Arthur, J. C. 1939. Manual of the rusts in the United States and Canada. Purdue Research Foundation, Indiana, 438 pp.

THE EFFECT OF STEMPHYLIUM LEAF SPOT COMPLEX ON YIELD OF FIELD-PLANTED BLUE LUPINE[1]

J. R. Edwardson, Homer D. Wells, and Ian Forbes, Jr.[2]

Abstract

The Stemphylium leaf spot complex of lupine, resulting from infection with virulent strains of S. botryosum Wallr. and S. solani Weber, has significantly reduced green weight and seed yields in field plantings of blue lupine. Lines that contain genes controlling resistance to the leaf spot complex have consistently produced significantly more seed and green weight than have susceptible lines of lupine.

INTRODUCTION

Commercial varieties of blue lupine (Lupinus angustifolius), used for forage and green-manure crops in the southeastern United States, are frequently attacked by Stemphylium solani (Weber) and S. botryosum (Wallr.) (3, 4). In the presence of these pathogens, resistant lines containing genes gl gl exhibit only scattered lesions while commercial varieties, all of which contain Gl Gl, exhibit a large number of lesions and are severely defoliated.

Virulent strains of S. solani and S. botryosum induce identical symptoms on susceptible varieties of blue lupine (3); however, certain introductions and a selection from commercial bitter blue lupine have been shown to be resistant to both pathogens (1, 2, 3). A selection (G. P.) from commercial bitter blue lupine, which contains the genes gl gl, has been increased to permit replicated testing in green-weight and seed yield. The recessive genes gl gl have been transferred from G. P. into a blue lupine forage line (60-206) (1, 2). The effect of the presence of genes controlling resistance to the Stemphylium leaf spot complex on field plot yields is described in this report.

YIELD COMPARISONS OF SELECTION G. P. AND COMMON BITTER BLUE LUPINE

Seed of the resistant selection G. P. and commercial bitter blue lupine were planted in randomized blocks at Gainesville, Florida. The blocks were replicated five times with each replication containing four 50-foot rows of each line. Each replication was bordered by single rows of the commercial variety. In 1960 and 1961 the yield tests were harvested during the last week in February when racemes had begun to develop. Ten-foot sections of each row, except the border rows, were selected at random. Plants in these sections were cut 3 inches above ground level and weighed in the field. Analysis of variance was applied to the green-weight data.

Highly significant differences between the green-weights of the two lines were found. The mean yields of the G. P. selection were 54,553 pounds/acre in 1960 and 31,043 in 1961, while those of the commercial bitter blue variety were 39,162 in 1960 and 20,791 in 1961. At the time of harvest in both years lesions occurred on some of the lower leaves of the G. P. selection while the commercial variety exhibited numerous lesions and was severely defoliated.

In 1960, S. botryosum alone was obtained from lesions in leaf specimens of both lines, whereas in 1961 both S. botryosum and S. solani were obtained.

SEED-YIELD COMPARISONS

Seed yields of the G. P. selection and commercial bitter blue lupine were compared in 1959, 1960, and 1961. Randomized blocks replicated five times, with each replication containing four 50-foot rows of each line, were used to obtain seed yields. Ten-foot sections of each

[1]Cooperative investigations at Gainesville, Florida and Tifton, Georgia of the Florida Agricultural Experiment Station, Gainesville, Florida and the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, the University of Georgia College of Agriculture Experiment Stations, and the Coastal Plain Experiment Station. Florida Agricultural Experiment Station Journal Series No. 1304.

[2]Associate Agronomist, Florida Agricultural Experiment Station, Gainesville, Florida; Pathologist and Agronomist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Tifton, Georgia, respectively.

row, except border rows, were selected at random. Pods in these sections were harvested by hand and threshed, and seed weights were obtained. Analysis of variance was applied to the seed-weight data. Highly significant differences were found between the seed weights of the two lines.

SEED-YIELD COMPARISONS IN POUNDS/ACRE

Line	1959	1960	1961
G. P. selection	608	243	790
Commercial bitter blue	214	85	29

Additional information concerning the influence of the Stemphylium leaf spot complex on seed yield was obtained in 1961. Seed yields in randomized blocks replicated four times were obtained from the green-manure varieties, commercial bitter blue and G. P., and from two forage varieties, Blanco and Borre lupine. Seed production was significantly reduced by the pathogens in the susceptible varieties. Commercial bitter blue produced 25 pounds of seed/acre, G. P. 786, Blanco 4.5, and Borre 9 pounds. The line 60-206, homozygous for the genes controlling resistance to the Stemphylium complex, a forage type similar to the Blanco variety, was planted in an eight-row seed increase nursery in 1961 at Gainesville. The mean yield obtained from these eight rows was 1134 pounds of seed/acre.

DISCUSSION

The presence of one or both of the pathogens of the Stemphylium complex at the time of harvest, together with the significantly larger yields exhibited by the resistant G. P. selection, indicates that the genes gl gl (1, 2) are primarily responsible for the observed increase in green-weight.

The problem of defoliation relatively late in the growing season in lupine varieties used for green-manure could be solved by turning them under before severe defoliation occurs. However, defoliation induced by the Stemphylium complex in forage varieties is a problem requiring a different solution. The use of resistant lines is the most readily available solution; such a line, 60-206, a white-flowered, white-seeded sweet selection resistant to the Stemphylium complex, is now being increased for more extensive evaluation.

Defoliation before and during seed maturation is primarily responsible for the reduction in seed yield of susceptible varieties of lupine. The use of the resistant lines, G. P. and 60-206, should enable growers to produce seed, green-manure, and forage lupines profitably in those areas of Georgia and Florida now infested with the Stemphylium leaf spot complex.

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FLORIDA AGRICULTURAL EXPERIMENT STATION, GAINESVILLE AND CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, TIFTON, GEORGIA

EFFECTS OF SOIL TEMPERATURE AND SELECTED CROP RESIDUES ON THE DEVELOPMENT AND SEVERITY OF FUSARIUM ROOT-ROT OF BEAN[1]

Charles R. Maier[2]

Summary

Fusarium root-rot of pinto bean was substantially influenced by different soil temperatures and selected crop residues in greenhouse tests. The disease developed most rapidly and was most severe at a constant soil temperature of 24°C; a temperature of 28° was least favorable to the disease. At constant soil temperatures of 18°, 24°, 28°, and 32° and at fluctuating temperatures ranging from 12° to 32°, the most rapid development of root-rot occurred during the first week after emergence; the greatest severity of root-rot occurred 3 weeks after emergence at all temperatures except 28°. The effects of seven crop residues on root-rot severity at the fluctuating temperatures were: barley, sorghum, and corn, suppressive; cotton, none; and tomato, alfalfa, and lettuce, increased severity. The conditions of residue and soil temperature most suppressive to root-rot were: barley at 32°C; sorghum at 28°; and corn at 28° and 32°. Under these conditions, the suppression of root-rot was not directly related to soil populations of the pathogen.

INTRODUCTION

Fusarium solani f. phaseoli (Burk.) Snyd. & Hans. is the major pathogen in a soil fungus complex inducing dry root-rot of pinto beans in New Mexico. Root-rot has grown steadily more prevalent over the past decade, and is currently a serious production factor. Largely because of this disease, bean rust, and drought, pinto bean acreage in the State has been drastically reduced. The pathogen infects beans most of the growing season, but symptoms are usually most severe during the late season. Dry root-rot affects primarily cortical tissue of the hypocotyl and larger roots, impairs water and solute uptake of infected plants (1).

Research on the effects of growing crops and crop residues on bean root-rot severity (2, 4, 5) have shown that certain residues high in C:N ratio are suppressive to the disease. These include barley straw (2, 5), soybean hay, sorghum, and Sudangrass (2). Lettuce, alfalfa, and tomato were found to increase disease severity (2). Since the disease has appeared most injurious to maturing beans, a study was undertaken to determine the influence of soil temperatures on the development and severity of root-rot in the presence of selected crop residues.

METHODS AND MATERIALS

In the greenhouse, constant soil temperature tanks were set at 18°, 24°, 28°, and 32°C, and a fluctuating temperature check, with a range of 12° to 35°, was located on an adjacent bench. Each tank held 16 small flats, necessitating two runs of the experiment for the four replications of eight residue treatments at each temperature.

Composted sandy loam soil, at pH 7.9, was mixed 2:1 with fine sand and sterilized in a soil autoclave. A culture suspension of the 10 most commonly occurring saprophytic fungi in their approximate recovery ratios was watered into the soil to establish a "normal" mycroflora. Residues of lettuce plants, tomato plants, green alfalfa hay, cotton plants, corn silage, sorghum silage, and barley straw ground in a small Wiley mill to particle size up to 4 mm were incorporated into certain portions of the soil. Each flat was then filled with 4.5 kg (10 pounds) of soil with appropriate residue amendment, and infested with F. solani f. phaseoli by watering with 50 ml of a conidial suspension adjusted to 10^5 macroconidia per ml. After 3 days' incubation, 50 bean seeds were planted per flat, and the flats placed at the appropriate temperatures. Emergence of the beans was virtually complete by 10 days after planting.

The influence of soil temperature was determined from samples of eight plants each removed at complete emergence and at four weekly intervals thereafter. Surviving plants were visually rated for root-rot severity on a scale of 0 to 4, where 0 means no infection to 4 means all plants severely rotted or dead. Tissue isolations were then made from infected plants to

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[2]Assistant Plant Pathologist, Agricultural Experiment Station, New Mexico State University, University Park, New Mexico.

establish F. solani f. phaseoli as the causal organism and to detect any invasion by Rhizoctonia solani.

Rhizosphere soil samples were collected as each plant sample was removed for estimating soil populations of the pathogen. Population estimates were obtained by a modified soil dilution technique. Rhizosphere soil containing small rootlets and organic debris was moistened and blended for 5 to 10 seconds in a Waring Blendor, then air-dried for 24 hours. One-gram portions of each sample were then diluted 1:100 in sterile sand, and a 1-gram portion of this dilution further diluted 1:100 in distilled water. The final 1 to 10^4 dilution was plated on potato-carrot-dextrose agar, six plates per sample. Fungi obtained were enumerated and transferred to sterile plates after 3 to 7 days, and identified after 5 to 7 days.

EXPERIMENTAL RESULTS

The seven residues, tested at the fluctuating temperature range, gave the following effects on Fusarium root-rot severity: barley, sorghum, and corn, suppression; cotton, no effect; and tomato, lettuce, and alfalfa, increased severity (Table 1). Barley straw strongly suppressed disease severity at all temperatures, but did not affect soil population of F. solani f. phaseoli (Table 2). Sorghum moderately reduced disease severity, but increased pathogen population in soil slightly. Corn reduced root-rot severity and pathogen soil population to a slight degree. Cotton produced no effect on disease severity, but increased soil pathogen population. Tomato, alfalfa, and lettuce increased both Fusarium root-rot and soil populations in ascending order.

Table 1. Effect of crop residue amendments on bean root-rot severity at a fluctuating temperature range of 12° to 35°C, 4 weeks after emergence.

Crop residue	Effect on severity	Magnitude of effect	Disease Severity rating[a]
Barley	suppressive	strong	1.2
Sorghum	suppressive	moderate	1.6
Corn	suppressive	slight	1.8
Cotton	none	------	2.2
CHECK (none)	-----	------	2.3
Tomato	increased	slight	2.7
Alfalfa	increased	moderate	2.9
Lettuce	increased	moderate	3.0
LSD .05			0.32

[a] Mean for four replications of eight plants each. Rating scale: 0- no infection; 1- slight discoloration, scattered lesions; 2- slight rotting; 3- moderate rotting; 4- severe rotting or death.

Table 2. Root-rot severity and Fusarium soil populations at four different constant soil temperatures as affected by eight soil amendments.

Crop residue	[a]Root-rot severity at: (°C)				[b]Fusarium population at: (°C)			
	18°	24°	28°	32°	18°	24°	28°	32°
Barley	1.5	1.3	1.1	1.0	2.3	2.1	2.5	2.6
Sorghum	1.9	1.8	1.5	1.3	3.0	3.0	3.2	3.3
Corn	2.2	2.1	1.6	1.6	1.9	2.1	2.3	2.2
Cotton	2.4	2.5	2.2	2.3	2.9	3.1	3.3	3.4
CONTROL	2.6	2.8	2.5	2.6	2.4	2.5	2.8	3.0
Tomato	2.9	3.0	2.4	2.7	3.0	3.2	3.1	3.4
Alfalfa	3.0	3.2	2.3	2.7	3.2	3.2	3.4	3.7
Lettuce	3.2	3.4	2.7	3.1	3.1	3.3	3.4	3.4
LSD .05	0.33	0.32	0.30	0.36	0.45	0.41	0.38	0.47

[a] Root-rot severity determined from four samples of eight plants by a 0 to 4 rating scale. Data are for 3 weeks after emergence.

[b] Soil populations of Fusarium estimated from 1-g samples, six plates per sample at a dilution of $1:10^4$.

Table 3. Root-Rot severity of bean plants taken at weekly intervals after emergence as affected by soil temperatures and selected crop residues. (eight plants per replicate).

Crop residue	Soil temperature	Root-rot severity for sample taken: (Weeks after emergence) Emer.	1	2	3	4
Barley	Fluc	0.4	1.0	1.2	1.4	1.2
	18°C	0.6	1.0	1.2	1.5	1.4
	24°	0.4	0.9	1.0	1.3	1.2
	28°	---	0.6	0.8	1.1	1.0
	32°	---	0.5	0.9	1.0	0.9
Sorghum	Fluc	0.6	1.2	1.6	1.8	1.6
	18°C	0.6	1.4	1.8	1.9	1.8
	24°	1.0	1.4	1.8	1.8	1.6
	28°	0.4	0.8	1.1	1.5	1.5
	32°	---	1.0	1.2	1.3	1.4
Corn	Fluc	0.8	1.2	1.5	1.7	1.8
	18°C	0.6	1.4	1.7	2.2	2.0
	24°	0.9	1.4	1.8	2.1	1.9
	28°	0.6	1.1	1.4	1.6	1.7
	32°	0.4	1.0	1.2	1.6	1.8
Cotton	Fluc	1.0	1.8	2.2	2.4	2.2
	18°C	0.8	1.6	2.1	2.4	2.3
	24°	0.6	1.4	2.0	2.5	2.4
	28°	0.8	1.4	1.8	2.2	2.3
	32°	0.6	1.5	1.9	2.4	2.4
CONTROL	Fluc	1.2	2.0	2.2	2.7	2.3
	18°C	1.0	1.8	2.3	2.8	2.5
	24°	0.9	1.6	2.2	2.8	2.7
	28°	0.8	1.6	2.0	2.3	2.5
	32°	1.0	1.8	2.2	2.4	2.4
Tomato	Fluc	1.4	2.2	2.7	2.9	2.7
	18°C	1.2	1.8	2.4	2.9	2.8
	24°	1.1	2.0	2.6	3.0	2.9
	28°	0.9	1.7	2.3	2.4	2.7
	32°	1.0	1.8	2.3	2.5	2.6
Alfalfa	Fluc	1.6	2.4	3.0	2.9	2.8
	18°C	1.2	2.0	2.4	3.0	2.9
	24°	1.4	2.4	2.9	3.2	3.1
	28°	1.0	1.7	2.3	2.3	2.5
	32°	0.9	1.4	1.9	2.5	2.8
Lettuce	Fluc	1.4	2.5	2.8	3.1	2.9
	18°C	1.0	2.1	2.6	3.2	3.1
	24°	0.8	2.0	2.4	3.2	3.3
	28°	1.0	1.7	2.2	2.5	2.8
	32°	1.3	1.9	2.5	3.1	3.0
LSD .05		0.20	0.29	0.32	0.30	0.37

LSD for Temp., at .05: Fluc, 0.32; 18°, 0.33; 24°, 0.41; 28°, 0.30; and 32°, 0.36.
Root-rot severity rating, 0 to 4 scale.

A constant soil temperature of 24°C was most conducive to severe disease for the various residue treatments, and 28° was least favorable (Fig. 1). The suppressive residues produced greatest and least severity, respectively, at the following temperatures: barley, 18° and 32°; sorghum, 18° and 28°; and corn, 18° and 28°, 32°. Root-rot severity in cotton amended soil was not influenced by different soil temperatures; the unamended control was similarly unaffected.

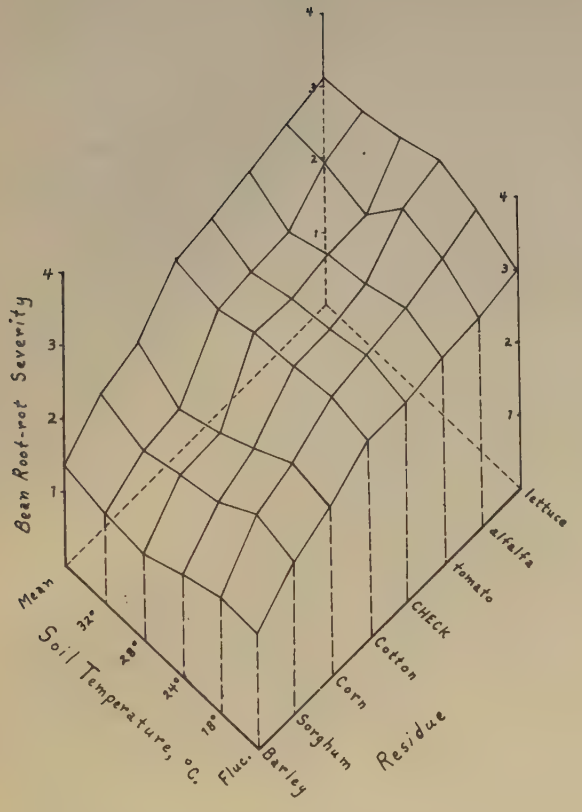

FIGURE 1. Root-rot severity of pinto beans incited by Fusarium solani f. phaseoli resulting from eight crop residue treatments at five different soil temperatures at 4 weeks after emergence.

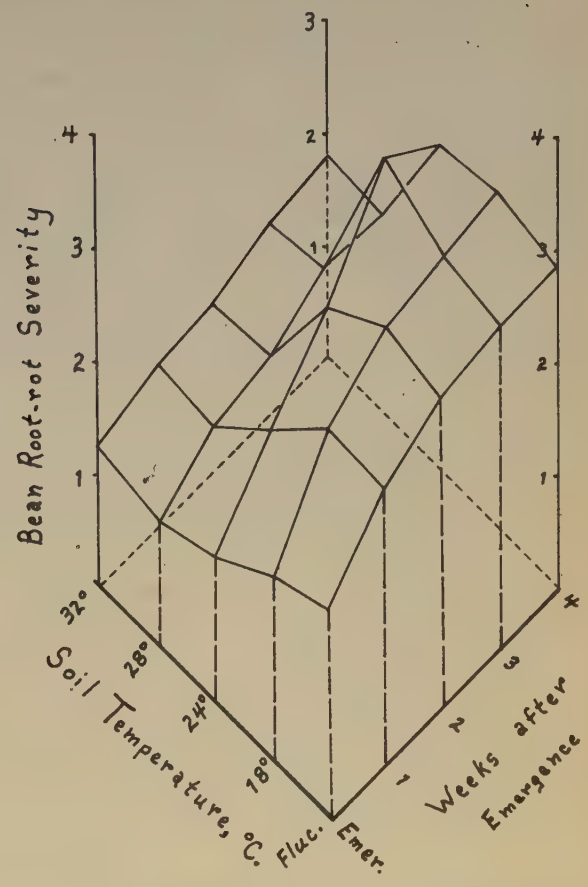

FIGURE 2. Root-rot severity at weekly intervals after emergence of pinto beans grown at five different soil temperatures. Data are the means of eight residue treatments.

Disease development at all temperatures for the organic amendments was most rapid during the first week after emergence, while for the unamended control, it was most rapid during the third week (Table 3). At 24°C, increases in disease severity for the mean of all residues remained relatively constant for 3 weeks; at all other temperatures, severity increases tapered off during the second week after emergence (Fig. 2). A composite of data for all residues showed that root-rot at 28° developed greatest severity at 4 weeks after emergence, but at 3 weeks at all other temperatures and in the fluctuating temperature check (Fig. 2).

Under the most favorable temperatures (28° and 32°) and suppressive residue substrated (barley and sorghum), bean root-rot severity suppression was not accompanied by reductions in soil populations of the bean Fusarium (Table 2).

DISCUSSION

Although the disease was more severe at relatively low temperatures in greenhouse experiments, root-rot is usually more prevalent in the field after mid-season when soil temperatures are relatively high. This inconsistency was not resolved, but two possible explanations are offered: 1) the seeding technique employed in this investigation may have excluded an important naturally-occurring fungus whose presence may have reversed the results; and 2) the apparent severity of field damage at higher temperatures may be exaggerated relative to earlier injury due to the more obvious symptoms. Early-season root-rot, from which many affected plants recover, would be obscure without more detailed investigation than mere field ob-

servation, upon which the seriousness of late season injury has been based.

The experimental results show that root-rot severity is substantially influenced by both soil temperatures and crop residues. Each residue treatment gave a unique response over the soil temperature range employed, but disease was generally less severe at the higher temperatures. The relationship of high soil temperature to root-rot suppression with certain organic amendments appears to be the stimulation of selective competition and antagonism by associated soil fungi. Why this should be so lies in the following considerations, based on this and a related study (3). Crop residues tend to break down more rapidly at high temperatures, hence the saprophytic activity of associated fungi would increase as favorable substrates became available. As saprophytic activity increased, more acute competition for substrate would occur, accompanied by some degree of antibiosis due to toxic metabolic or staling products of the interacting fungi. The infection process as well as the growth of the bean Fusarium would be retarded by these interactions. That this occurs is suggested by the lack of correlation between the reduction in disease severity at all temperatures and the nil effect or increase of pathogen soil populations by barley and sorghum amendments.

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NEW MEXICO AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY PARK, NEW MEXICO

INFLUENCE OF VARIOUS NITROGEN AND LIGHT SOURCES ON SOME CULTURAL CHARACTERS OF FUSARIUM OXYSPORUM F. LYCOPERSICI

A. Mahadevan and Nestor E. Caroselli[1]

Abstract

Fusarium oxysporum f. lycopersici (Sacc.) Snyd. & Hans. was grown in a modified Czapek's agar medium with 0.1, 0.3, 0.5 and 1.0% each of asparagine, urea, ammonium nitrate, potassium nitrate, sodium nitrate and calcium nitrate and under four different light conditions: 1) laboratory condition, 2) continued darkness, 3) continued light and, 4) alternating 24 hours light and 24 hours darkness at 20° C. The fungus behavior in each concentration was studied over a 12-day period. Both concentration and source of nitrogen under different light conditions influenced the daily linear growth, zonation, pigmentation and sporulation of the fungus.

Different environments have been shown to have a profound influence on the cultural characteristics exhibited by various species of Fusarium (1, 2, 3, 4, 5, 6).

In this investigation experiments have been conducted to determine the influence of various sources and levels of nitrogen on the behavior of the tomato wilt fungus, Fusarium oxysporum f. lycopersici grown under various periods of light and dark.

METHODS AND MATERIALS

Czapek's agar medium in which test compounds were substituted for the sodium nitrate was employed throughout the investigation. Sterile Petri dishes containing 20 ml of the medium were inoculated with 1-cm disc of 14-day-old fungus culture grown on PDA and incubated at room temperature. Three replicates of three Petri dishes each were used for each test. Four different levels (0.1, 0.3, 0.5, and 1.0%) of six nitrogen compounds were used. Two organic sources were L-asparagine and urea while the inorganic compounds were ammonium nitrate, potassium nitrate, sodium nitrate and calcium nitrate. The fungus was grown under the following conditions: 1) laboratory condition[2], 2) continued darkness, 3) continued light and, 4) alternating 24 hours light and 24 hours darkness. A 600-foot candle power fluorescent light was used as the source of light in test 3) and 4) above. All the Petri dishes were inverted and incubated at 20° to 21° C; growth was recorded daily up to the twelfth day.

A check with Czapek's medium without any nitrogen source was used throughout the investigation.

In these tests the term sporulation indicates the presence of microconidia. Also poor, moderate and heavy sporulation refer to 1-600, 600-1200 and above 1200 spores per field respectively.

DISCUSSION OF RESULTS

The fungus differed in certain cultural characteristics (that is, linear growth, matting, pigmentation, staling and sporulation) when grown in the presence of different sources and levels of nitrogen as well as various light and dark conditions (Table 1).

Although linear growth varied in all sources of light, darkness, and nitrogen content, the most striking fact was a decrease in growth with an increase in the concentration of both asparagine and urea regardless of the length of darkness or light (Table 1).

Differences in the density of the fungus mat were also observed. On media containing the different levels of asparagine and urea the fungus produced a dense mat under conditions of alternating light and dark and in continued darkness. With urea, poor and moderately dense mat formation occurred under conditions of constant light and alternating light and darkness respectively. Asparagine stimulated only moderately dense mat surfaces in all concentrations under continued light as well as alternating light and darkness.

The density of the mat increased when the concentrations of the nitrogen in the form of calcium nitrate were increased under all conditions of light. When potassium and ammonium nitrate were used, the same tendency was noted in all conditions, except under continued darkness, where a dense and poor matting resulted in low (0.1 and 0.3%) and high (1.0%) concentrations respectively.

In the presence of sodium nitrate the fungus mat was dense in concentrations of 0.1 and 0.3%. Conversely, a very poor mat was formed with an increase of sodium nitrate regardless of the conditions.

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[1]Graduate assistant in Botany and Professor of Botany, respectively, University of Rhode Island.

[2]Refers to normal light and dark each day.

Table 1: Average growth in centimeters of Fusarium oxysporum f. lycopersici (Sacc.) Snyd. & Hans. grown on various sources and levels of nitrogen under laboratory conditions (R), continued darkness (D), continued light (L) and alternating periods of 24 hours' darkness and 24 hours' light (A).

%	Incubation (Days)	Asparagine R	Asparagine D	Asparagine L	Asparagine A	Urea R	Urea D	Urea L	Urea A	Ammonium Nitrate R	Ammonium Nitrate D	Ammonium Nitrate L	Ammonium Nitrate A	Potassium Nitrate R	Potassium Nitrate D	Potassium Nitrate L	Potassium Nitrate A	Sodium Nitrate R	Sodium Nitrate D	Sodium Nitrate L	Sodium Nitrate A	Calcium Nitrate R	Calcium Nitrate D	Calcium Nitrate L	Calcium Nitrate A	Check
0.1	1																									
	2	2.1	2.0	1.6	2.1	1.7	1.9	1.1	1.7	2.3	2.6	2.6	2.6	2.7	2.7	2.3	2.5	1.8	2.1	2.1	2.0	2.9	2.8	2.6	2.9	Scanty
	3	3.3	3.5	3.1	3.4	3.0	3.1	1.6	2.2	3.1	3.2	3.7	3.5	4.0	4.0	2.9	3.3	3.1	3.4	3.4	3.2	4.3	4.2	3.1	3.7	growth;
	4	5.0	4.9	4.8	4.0	4.3	4.0	2.2	2.9	4.6	4.1	4.7	4.4	5.3	5.3	3.6	4.4	4.7	4.6	4.5	4.3	5.7	5.6	3.7	5.0	micro-
	5	6.0	5.7	5.5	5.7	5.6	4.9	2.6	3.6	5.6	4.7	5.7	5.2	6.7	6.7	4.3	5.1	6.0	5.9	5.5	5.5	7.0	6.9	4.2	5.6	conidia
	6	7.3	6.8	6.4	6.8	6.8	5.9	3.2	4.7	6.8	5.5	6.7	5.8	7.9	8.7	4.7	6.0	7.3	7.0	6.9	7.0	7.9	8.2	4.6	6.6	rarely
	7	8.7	8.0	7.5	8.0	8.1	7.2	3.8	5.1	7.7	6.3	7.5	6.5	8.7	Com.[a]	5.3	7.2	8.7	8.2	8.1	8.0	8.5	8.7	5.7	7.4	produced
	8	Com.	Com.	Com.	Com.	Com.	8.2	4.0	5.5	8.7	7.3	8.2	7.7	Com.		5.8	7.8	Com.	Com.	Com.	Com.	Com.	Com.	6.8	8.3	
	9						Com.	4.1	5.8	Com.	8.2	Com.	8.5			7.3	8.6							7.5	8.6	
	10							4.1	6.2		Com.		Com.			7.8	Com.							Com.	Com.	
	11							4.3	6.6							8.3										
	12							4.5	7.1							Com.										
0.3	1																									
	2	2.0	2.0	1.5	2.0	1.2	1.5	1.0	1.2	2.4	2.4	2.5	2.6	2.8	2.7	2.0	2.5	2.0	2.1	1.9	2.0	2.8	2.7	2.8	2.6	
	3	3.3	3.5	3.1	3.2	2.4	2.6	1.4	1.9	3.3	3.3	3.4	3.4	4.1	4.0	2.7	3.2	3.4	3.3	3.2	3.1	3.9	3.8	3.2	3.6	
	4	4.9	5.0	4.5	4.6	3.7	3.8	1.8	2.7	4.5	4.4	4.6	4.4	5.6	5.4	3.4	4.6	4.6	4.4	4.2	4.4	5.3	5.0	3.7	4.9	
	5	5.8	5.8	5.4	5.3	5.0	4.7	2.1	2.7	5.3	5.4	5.5	5.1	6.9	6.7	3.9	5.1	5.8	5.5	5.3	5.6	6.5	6.2	4.0	5.4	
	6	6.9	6.9	6.3	6.1	6.2	5.8	2.7	4.1	6.3	6.6	6.4	5.9	8.0	8.1	4.5	6.1	7.4	7.0	6.5	6.9	7.6	7.3	4.6	6.1	
	7	8.2	8.0	7.4	7.3	7.4	6.9	2.9	4.6	7.2	7.3	6.9	6.7	Com.	Com.	4.9	7.0	8.2	8.3	7.6	8.0	8.6	8.5	5.6	7.6	
	8	Com.	Com.	8.4	8.0	8.3	8.0	3.2	5.1	8.1	7.9	7.9	7.1			6.3	7.9	Com.	Com.	8.6	Com.	Com.	Com.	6.6	8.3	
	9			Com.	Com.	Com.	Com.	3.3	5.4	8.6	8.6	8.4	7.9			6.9	8.4			Com.				7.2	Com.	
	10							3.3	5.8	Com.	Com.	8.8	8.2			7.9	Com.							8.1		
	11							3.7	6.1			Com.	8.8			8.1								Com.		
	12							3.9	6.8				Com.			8.5										
0.5	1																									
	2	2.0	1.9	1.6	2.0	1.2	1.1	1.0	1.1	2.3	2.2	2.5	2.5	2.6	2.7	1.9	2.4	2.1	1.9	1.8	1.8	3.2	3.3	3.4	3.5	
	3	3.3	3.3	2.9	3.3	2.9	2.5	1.0	1.9	3.2	3.2	3.4	3.4	3.9	4.1	2.8	3.4	3.3	3.2	3.0	3.0	4.7	5.0	4.3	4.8	
	4	4.9	4.8	4.4	4.7	3.7	3.4	1.3	2.7	4.3	4.3	4.5	4.3	5.3	5.5	3.5	4.6	4.7	4.4	4.1	4.0	6.0	5.8	5.0	5.1	
	5	5.7	5.3	4.8	5.4	4.9	4.3	1.6	3.1	5.1	5.3	5.4	5.0	6.5	6.8	4.2	5.2	6.0	5.6	5.3	5.2	7.2	6.5	5.2	5.5	
	6	6.9	6.8	5.7	6.4	6.0	5.5	1.9	4.2	6.2	6.3	6.1	5.7	7.8	8.1	5.3	6.4	7.2	7.0	6.5	6.6	7.9	7.8	5.8	6.8	
	7	8.1	7.2	7.0	7.6	7.3	6.7	1.9	4.1	7.2	7.3	6.9	6.7	Com.	Com.	6.4	7.4	8.5	8.1	7.6	7.2	8.8	8.6	6.2	7.8	
	8	Com.	8.6	8.0	8.2	8.2	7.8	2.1	5.5	8.2	8.3	7.7	7.2			7.4	8.6	Com.	Com.	8.8	Com.	Com.	Com.	7.0	8.4	
	9		Com.	Com.	Com.	Com.	Com.	2.1	5.7	Com.	8.9	8.6	7.7			8.0	Com.			Com.				8.0	Com.	
	10							2.2	6.0		Com.	Com.	8.3			Com.								8.6		
	11							2.2	6.5				8.7											Com.		
	12							2.3	6.7				8.8													
1.0	1																									
	2	2.0	1.9	1.7	2.0	1.1	1.0	1.0	1.1	2.1	2.1	2.4	2.3	2.8	2.7	2.3	2.6	1.8	1.8	1.8	1.8	2.6	2.8	3.0	3.1	
	3	3.3	3.2	2.9	3.3	1.6	1.9	1.0	1.5	3.2	3.0	3.4	3.1	4.2	4.0	3.4	3.8	3.1	3.1	3.1	3.0	3.8	3.9	3.6	4.8	
	4	4.9	4.8	4.4	4.9	2.8	2.8	1.0	1.8	4.0	4.0	4.4	4.2	5.5	5.4	4.2	5.1	4.3	4.2	4.3	4.2	5.5	5.4	3.7	5.1	
	5	5.7	5.4	4.8	5.3	3.7	3.6	1.4	2.3	4.9	5.1	5.2	5.0	6.9	6.7	5.3	5.8	5.5	5.4	5.2	5.1	6.4	6.2	4.0	5.5	
	6	6.7	6.4	5.3	6.3	4.2	4.4	1.7	2.5	5.9	6.0	6.2	5.9	8.1	8.1	6.2	7.1	6.7	6.5	6.5	6.2	7.5	7.6	4.9	6.8	
	7	7.3	7.1	5.9	7.0	4.4	4.8	1.8	2.7	6.8	7.0	7.0	6.5	Com.	Com.	7.1	8.0	7.9	7.7	7.4	7.2	8.3	8.5	6.1	7.8	
	8	7.7	7.4	6.7	7.8	4.6	4.9	1.8	3.0	7.6	7.8	8.4	7.1			7.9	Com.	Com.	8.8	8.7	8.6	Com.	Com.	6.9	8.4	
	9	8.3	8.1	7.2	8.1	4.7	5.0	1.8	3.0	8.4	8.6	8.7	7.6			8.6		Com.	Com.	Com.				8.0	Com.	
	10	8.4	8.4	7.5	8.3	4.9	5.1	1.8	3.1	Com.	Com.	8.7	8.1			Com.								Com.		
	11	8.7	8.7	7.9	8.7	5.2	5.2	1.9	3.2			8.8	8.2													
	12	Com.	Com.	8.1	8.9	5.5	5.2	1.9	3.2			8.9	8.3													

[a] Com. means Petri dish completed by the fungal mat.

Production of a diversity and intensity of pigment appears to be influenced by the concentration as well as the source of nitrogen and the amount of light. Low concentration (0.1%) of urea favored the production of a violet color, while with increasing concentrations of urea, the pigment changed to yellow. A similar relationship was noted when asparagine was used.

The intensity of pigment increased when the concentration of calcium nitrate was increased in the laboratory conditions where yellow pigmentation was evident, while in constant light the pigment attained a purple, purple-yellow and yellow at concentrations of 0.1%, 0.3% and 1.0% respectively. The fungus exhibited a similar relationship in alternating light conditions, while the absence of light favored the production of yellow pigment which diminished in intensity at high concentrations; thus at 1.0% no observable pigment was noted. Regardless of the concentration of potassium nitrate, a creamy to orange-yellow pigment was observed under laboratory condition, constant light and continued darkness.

In the presence of ammonium nitrate a light yellow undersurface occurred at all concentrations in laboratory condition. Constant light favored the production of a deep violet color at low concentration (0.1%) but as the concentration increased the intensity of pigment decreased and at 1.0% it was yellow with a tinge of purple. There was no pigment noted when cultures were grown under alternating light condition. Under continued darkness, low concentration (0.1%) favored the production of a deep violet pigment that formed on the periphery and enclosed a creamy-white zone.

The aerial mycelium grown under alternating light and dark periods produced alternating bands of thick and thin growth respectively, regardless of the source and concentration of nitrogen. This phenomenon is probably due to accumulation of staling products.

Although some staling was noted no general conclusion could be drawn regarding the effect of nitrogen source on staling under the conditions of this experiment.

The rate and nature of sporulation was affected by the concentration as well as by the source of nitrogen in various light conditions. At concentrations of 0.1 and 0.3% asparagine and urea favored heavy microconidial production. Higher concentrations (0.5 and 1.0%) of these chemicals promoted only moderate production of microconidia under ordinary light conditions, but in continued light there was a definite increase in sporulation with an increase in concentrations of asparagine and urea. The fungus grown on these media also produced an abundance of macroconidia.

Regardless of the light conditions, sporulation was heavy in all concentrations of ammonium and sodium nitrates. High concentrations (0.5 and 1.0%) of both potassium and calcium nitrates stimulated sporulation. Heavy sporulation occurred when potassium nitrate was used in a very low (0.1%) concentration under laboratory condition, while moderate production was noticed in other light conditions. Calcium nitrate (0.1%) favored poor sporulation in all light conditions. Heavy spore production was recorded in media containing 0.3% concentration of potassium nitrate. A similar concentration of calcium nitrate resulted in moderate sporulation in laboratory conditions but other light conditions stimulated heavy sporulation.

With the exceptions of ammonium and sodium nitrate (0.1 and 0.3%) in alternating light and darkness, nitrogen sources did not induce the production of microconidia. However, these concentrations favored a dense mat.

In the control plates fungus growth was scanty and rarely produced microconidia.

These results, noted under the conditions of this investigation, indicate the effect of various environmental factors on the multiplicity of characters displayed by this fungus.

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DEPARTMENT OF BOTANY, UNIVERSITY OF RHODE ISLAND, KINGSTON, RHODE ISLAND

INFLUENCE OF RESISTANT VARIETIES ON VIRULENCE LEVEL WITHIN NATURAL POPULATIONS OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE

J. L. Apple

Abstract

Several isolates of Phytophthora parasitica var. nicotianae were collected from each of 16 locations. Isolates from 10 locations had been exposed to black shank resistant tobacco varieties for 1 to 3 years and the isolates from the other 6 locations had been exposed only to susceptible varieties. Isolates from each location were checked for virulence in greenhouse tests on both resistant and susceptible varieties. In general, isolate groups from locations planted to resistant varieties had a higher virulence index than corresponding isolates from locations planted only to susceptible varieties. These data support the hypothesis that the population virulence level is increased by adaptive selection in response to plantings of black shank resistant varieties.

INTRODUCTION

Natural populations of Phytophthora parasitica (Dast.) var. nicotianae (Breda de Haan) Tucker, causal agent of the black shank disease of tobacco, are comprised of many strains that vary in virulence. Commercially available black shank resistant tobacco varieties possess genetic resistance of a quantitative type. When planted in infested soil, these varieties usually provide adequate disease control, especially when used in a rotation system with other crops; however, root infection in these varieties is common although aboveground symptoms may not be evident. Thus, the fungus likely maintains a high inoculum potential in the presence of resistant varieties. It has been postulated that successive plantings of such black shank resistant tobacco varieties in infested soil would exert a selection pressure in favor of the highly virulent biotypes (1). The result would be population dominance by highly virulent types and the eventual exclusion of the less virulent types. Also, under these conditions, mutation pressure would exist toward higher virulence.

Limited evidence obtained previously supported this hypothesis (1). The object of the present study was to explore the problem more thoroughly and systematically and to determine if adaptation toward higher population virulence has occurred in response to repeated plantings of resistant varieties in infested soil.

METHODS AND MATERIALS

Six to ten isolates of the black shank fungus were collected from each of 16 fields during the summer of 1957 (Table 1). Eash isolate came from an individual plant and was placed into one of two classes, depending on the disease and varietal history of the collection site:

Class "S" - Isolates from fields planted in a susceptible variety and in which black shank first appeared in 1957 or fields that were infested prior to 1957 but in which resistant varieties had not been grown. Thus, these isolates represented an "unselected" population insofar as any selection pressure imposed by resistant varieties.

Class "R" - Isolates from fungus population exposed to selection pressure of a resistant variety for 1 to 3 years. Field may have been planted to susceptible variety in 1957 but following the use of resistant varieties in previous years. In the latter case, it was assumed that diseased plants of the susceptible variety yielded a valid sample of a previously "selected" population.

Isolates from 10 locations were classified as "R" and from 6 as "S" prior to their evaluation for virulence in greenhouse tests. The screening technique and indexing system used were described previously (1). The virulence index is based on the number of diseased plants per day over the 12-day period following inoculation. An index of 10 would indicate all plants dead on the third day after inoculation, and an index of 0 would indicate no disease development during the 12-day period following inoculation. The tobacco varieties used were Bottom Special, black shank susceptible, and Coker 139, highly resistant. The different groups of class "S" and class "R" isolates were selected and paired at random. In each inoculation test, one to three groups from each class were tested, using five to nine isolates from each group.

Table 1. Source of isolates and disease and cropping history of collection sites.

Location	County	Classification	Cropping history by year[a]			% estimate of diseased plants (1957)	Year black shank first observed in field
			1955	1956	1957		
1	Robeson	R	Tobacco (R)	Corn	Tobacco (S)	75	1954 (?)
4	do.	S	do. (S)	do.	do. (S)	75	1957
6	Wilson	R	do. (R)	Tobacco (R)	do. (S)	15	1955 (?)
8	Pitt	R	do. (R)	Wheat	do. (S)	60	1950
9	Franklin	S	do. (S)	Fallow	do. (S)	95	1955
12	do.	S	Corn	Corn	do. (S)	75	1957
13	Wake	R	Tobacco (R)	Tobacco (R)	do. (S)	80	1955
16	Beaufort	R	do. (R)	Corn	do. (R)	75	1955
17	do.	R	do. (R)	do.	do. (R)	30	1955
21	Martin	R	do. (R)	Tobacco (R)	do. (S)	65	1954 (?)
22	Yadkin	S	do. (R)	do. (R)	do. (S)	3	1957
23	do.	R	do. (R)	do. (R)	do. (R)	85	1955
24	Forsyth	R	do. (R)	do. (R)	do. (S)	75	1955
26	Haywood	S	do. (S)	do. (S)	do. (S)	25	1956
27	do.	S	do. (S)	do. (S)	do. (S)	20	1956
28	Pitt	R	do. (R)	Oats	do. (R)	35	1953

[a]Where tobacco occurs in the cropping history, the parenthetical letter indicates black shank susceptible (S) or resistant (R) variety.

The data were analyzed as a split-plot with isolates as the wholeplot variable and variety as the sub-plot. The degrees of freedom and sums of squares for isolates were subdivided for the individual isolate locations. The pooled error term (Error A) was used in all cases to test location significance since the individual group error terms were homogeneous as determined by Bartlett's test of homogeneity (4).

RESULTS AND DISCUSSION

Isolate groups of class "R" generally had a higher virulence index than those of class "S". Typically, within group isolate variability of class "S" populations was greater than that of class "R" populations as evidenced by the significant difference between isolates from location 4 but no significance between virulence indices of isolates from location 16 (Tables 2 and 3). The difference between location means in test 1 was highly significant. This average difference was due to the consistently high virulence indices for isolates from location 16. Some isolates from the unselected population (location 4) had a virulence index as high as any from location 16; however, the weakly virulent types in the latter presumably were eliminated from the population by selection imposed by resistant varieties.

Test 1 was repeated using the same isolates (Table 2). The combined analysis of variance for the two tests lends support to the validity of this screening method. The mean square for tests was highly significant due to the generally higher virulence indices in test 1, but they were disproportionately higher on the resistant variety, giving a significant "Tests x Varieties" interaction. Of more importance, however, is the fact that "Varieties x Isolates" and "Tests x Isolates" were not significant. This indicated that individual isolates maintained the same relative position in both tests across both varieties. Based on these data, subsequent screening tests were not repeated.

Isolates from six locations (three each of classes "R" and "S") were compared in test 3 (Table 4). Isolates from location 22 ("S") had a significantly lower virulence index than all others. The other two groups of class "S" were not significantly different from either of the three groups of class "R". Tests 4 and

Table 2. Combined analysis of variance for tests 1 and 2.

Source	d. f.	ss	ms	F
Total	287	2,185.0		
Replications	3	27.5	9.2	4.44
Isolates	17	293.4	17.2	8.31 **
Location 4 ("S")	(8)	(223.5)	(27.9)	14.16 **
Location 16 ("R")	(8)	(27.9)	(3.5)	1.64
Between locations	(1)	(42.0)	(42.0)	16.80 *
Reps x isolates (Error A)	51	105.9	2.07	
Varieties	1	842.4	842.4	623.97 **
Var. x isolates	17	43.2	2.5	1.88
Var. x reps x isol. (Error B)	51	69.2	1.35	
Tests	1	441.5	441.5	228.75 **
Tests x isolates	17	40.2	2.4	1.22
Tests x varieties	1	22.0	22.0	11.39 **
Tests x var. x isol.	17	49.9	2.9	1.52
Error C	108	208.7	1.93	

*Significant at 5%.
**Significant at 1%.

Table 3. Combined virulence indices from tests 1 and 2, comparing isolates of Phytophthora parasitica var. nicotianae from two locations.

Isolate no.	Virulence index by variety: Bottom Special	Coker 139	Isolate mean[a]
	Location 4 ("S")		
800	4.6	1.6	3.1
801	6.5	2.7	4.6
804	3.4	0.6	2.0
807	5.4	2.3	3.8
810	5.8	3.5	4.6
811	5.1	1.5	3.3
813	6.1	2.6	4.4
814	1.7	0.0	0.9
816	6.1	3.2	4.6
Location means[b]	5.0	2.0	3.5
	Location 16 ("R")		
1012	5.3	1.8	3.5
1013	5.6	2.5	4.0
1017	6.0	1.9	4.0
1018	7.1	2.2	4.7
1019	6.0	2.1	4.0
1021	7.4	2.7	5.1
1022	6.0	2.8	4.4
1023	5.9	2.2	4.0
1026	6.5	2.6	4.6
Location means [b]	6.2	2.3	4.2

[a]LSD between isolate means: 5% = 1.0; 1% = 1.4.
[b]LSD between isolate means between locations: 5% = 0.34; 1% = 0.45.

5 each compared the virulence of isolates from four locations, three of class "R" and one of class "S". In test 4, the virulence index for location 12 ("S") was significantly less than the class "R" isolates (Table 4). In test 5, the virulence index for location 9 ("S") was significantly less than that for two of the "R" locations but not different from the third (Table 4).

Thus, in testing isolates from 6 "S" locations and 10 "R" locations, all but three groups so classified behaved as postulated. In test 3, isolates from locations 26 and 27 (Class "S") had virulence indices as high as the "R" groups. These isolates were from Haywood County in western North Carolina and of the burley strain (1). This strain is distinguishable from the flue-cured strain in culture, and there has been some suggestion that it is more aggressive in

Table 4. Mean virulence indices of isolates of Phytophthora parasitica var. nicotianae from different locations.

Location	Classification	Virulence index by variety: Bottom Special	Coker 139	Location mean
		Test 3[a]		
22	S	4.7	1.7	3.2
26	S	7.3	3.8	5.5
27	S	8.2	4.7	6.5
13	R	7.8	4.0	5.9
24	R	8.0	3.8	5.9
28	R	8.2	4.3	6.3
		Test 4[b]		
6	R	5.6	1.3	3.4
8	R	5.1	1.2	3.2
12	S	2.8	0.7	1.7
23	R	5.4	1.1	3.3
		Test 5[c]		
1	R	3.2	1.8	2.6
9	S	1.8	1.0	1.4
17	R	3.0	1.2	2.1
21	R	1.4	1.0	1.2

[a]Six isolates tested from each location; LSD between location means: 5% = 0.67; 1% = 0.89.
[b]Five isolates tested from each location; LSD between location means: 5% = 0.50; 1% = 0.72.
[c]Five isolates tested from each location; LSD between location means: 5% = 0.7.

plants carrying Florida 301 type resistance than the flue-cured strain (3). These isolates of the burley strain were collected as representative of an unselected population; however, if they represent a physiologically specialized form, their comparison with the flue-cured isolates was not valid. The other deviation from the expected was location 21 (Table 4). The virulence of this group of isolates, classed as "R", was significantly less than the two "R" groups and not different from the single "S" group in the same test. There is no apparent explanation for the relatively low virulence of this group. They come from a field presumably infested before 1957 and planted to a susceptible variety in 1957 but which had been planted to resistant varieties for the three previous years.

These data support the hypothesis that repeated use of resistant varieties with the Florida 301 type resistance will cause selection and perpetuation of highly virulent types and eventual exclusion of lesser virulent types.

It is assumed that the phenomenon observed was adaptive selection from a mixture of population genotypes rather than the evolution of a higher degree of virulence through mutation or host passage (2).

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DEPARTMENT OF PLANT PATHOLOGY, NORTH CAROLINA STATE COLLEGE, RALEIGH, NORTH CAROLINA

EFFECTIVENESS OF TREATMENTS FOR THE CONTROL OF SOIL-BORNE FUSARIUM INFECTIONS OF MANALUCIE TOMATOES[1]

Huey I. Borders[2]

Abstract

Various materials were tested during the tomato-growing season of 1960-1961 for control of a new pathogenic race of Fusarium attacking hitherto resistant tomato varieties.

Two materials, a copper sulfate-captan-PCNB compound and 2-chloro-3-(tolyl-sulfonyl) propionitrile, yielded statistically significant reductions in Fusarium infections.

INTRODUCTION

During the spring of 1960, Fusarium wilt resistant tomato varieties, including Manalucie, were found by Stall[3] to be infected with a new pathogenic race of Fusarium (the Delray isolate of Fusarium oxysporum f. lycopersici (Sacc.) Snyd. & Hans.).

From October 1960 to May 1961 a series of tests was carried out to test materials that might eradicate or control the new race of Fusarium. The results of the first season's tests are reported in this paper.

METHODS AND MATERIALS

Experiments were conducted on soil infested with the new pathogenic race of Fusarium on the farm where it was found. All tests were set up in randomized blocks, replicated four times. Materials were applied as in-furrow treatments to the beds, as broadcast treatments, or as soil drenches before planting. Manalucie tomato seeds were field-seeded on beds with four-and-one-half foot centers and were thinned to about 10 inches apart in the row. The vines were pruned to a single stem and were "staked" by training them on cord which was tied to an overhead wire. All plots received the same fertilization, foliar fungicides and insecticides.

Every 10 to 14 days all plants in the tests were examined for the presence of Fusarium by making shallow incisions on the stems, beginning at ground level. Sufficient cuts were made to insure checking all the vascular bundles in each plant for discoloration. Cultures of samples from discolored vascular tissue yielded a Fusarium[4].

RESULTS AND DISCUSSION

EF 1261-960 applied at 39.2 pounds/acre resulted in significant differences at the 1% level in comparison with the untreated check (Table 1). It reduced the Fusarium infections to 3% of the total plants in the treated plots as compared with 20% in the untreated checks. In this experiment Manalucie tomato seeds were planted 16 November 1960. The treatments were applied as drenches on 7 December 1960, when the tomato seedlings were about 3 inches high. Counts were made 4 April 1961.

At a smaller dosage rate of only 9.8 pounds/acre (Table 1), treatments with EF 1261-960 were effective at the 5% level of significance. Infected plants were reduced to 7% as compared with 20% in the untreated check plots.

Monsanto 30249 at a dosage rate of 2 pounds active ingredient per acre reduced Fusarium infections to 6% (Table 1).

In another experiment, K-6, while not producing a statistically significant reduction in the amount of Fusarium infections, did cause a plant stimulation that persisted throughout the growing season (Table 2). The plants that received an in-furrow treatment with K-6 at approximately 40 pounds active per acre kept their lush green color throughout the season and were

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[1]Florida Agricultural Experiment Station Journal Series, No. 1300.

[2]Associate Plant Pathologist, Everglades Experiment Station, Plantation Field Laboratory, Fort Lauderdale, Florida.

[3]Stall, Robert E. 1961. Development of Fusarium wilt on resistant varieties of tomato caused by a strain different from race 1 isolates of Fusarium oxysporum f. lycopersici. Plant Disease Reptr. 45: 12-15.

[4]The author thanks Dr. Robert E. Stall for identifying material from this experiment as containing the new Delray isolate of Fusarium.

Table 1. Effect of various materials for control of the new pathogenic race of Fusarium (Delray isolate) on Manalucie tomatoes.

	Treatment	Number of plants (means)	Number of diseased plants (means)	% diseased plants (means)
1.[a]	EF 1261-960, 9.8 pounds/acre	45	3	7
2.	EF 1261-960, 19.6 pounds/acre	43	4	9
3.	EF 1261-960, 39.2 pounds/acre	41	1	3
4.	EF 1261-960, 58.8 pounds/acre	46	3	7
5.[b]	K-6, approx. 1 pint of .1 active/acre	43	7	16
6.	K-6, approx. 1 quart of .1 active/acre	42	6	13
7.	K-6, approx. 2 quarts of .1 active/acre	45	9	20
8.	K-6, approx. 3 quarts of .1 active/acre	43	6	14
9.	K-6, approx. 4 quarts of .1 active/acre	47	5	10
10.[c]	Monsanto 30249, 2 pounds active/acre	44	3	6
11.	Monsanto 30249, 1 pound active/acre	47	5	11
12.	Check - no treatment	46	9	20
	LSD between means at 5%	NS	6	13
	LSD between means at 1%		8	17

[a] EF 1261-960=Copper sulfate-captan-PCNB compound furnished by Everglades Fertilizer Co., Ft. Lauderdale, Florida

[b] K-6= Hexachlorophene solution furnished by Nationwide Chemical Co., Ft. Myers, Florida.

[c] Monsanto 30249=2-chloro-3-(tolylsulfonyl) propionitrile furnished by Monsanto Chemical Co., St. Louis, Missouri.

Table 2. Plant stimulation effect and disease incidence observed in one of the Fusarium control experiments.

Treatment	Total number plants	Number Fusarium infected	Green weight of 10 plants: total weight (pounds)	Green weight of 10 plants: means	% diseased plants
K-6 approx. 40 pounds active/acre	249	22	118.4	30	9
Monsanto 30249, 2 pounds active/acre	282	39	83.3	21	14
K-6 approx. 20 pounds active/acre	316	56	64.8	16	18
Check - no treatment	283	82	57.9	15	29
LSD 5%		NS		14	NS

still bearing marketable tomatoes 2 weeks after the other plants in the same experiment had either died or had turned yellow and ceased to bear marketable fruit. This effect was achieved in spite of the fact that all plants in the experiment were heavily infested with root-knot nematodes. All treatments were applied as in-furrow soil treatments at time of planting. Manalucie variety seeds were field-seeded immediately after soil treatments were applied. Seeds were planted 21 October 1960. The counts were made and weights taken on 23 March 1961. Total plants and total Fusarium infected plants were taken from counts made of all Manalucie variety tomato plants in two 50-foot plant rows. Green weights were derived by cutting every third plant for a total of 10 plants from the middle row of each plot at a height of 20 inches from the ground and weighing the tops. This included the fruit on the plants.

Other materials and application rates will be tried during the 1961-1962 growing season. Plans also include further work with EF 1261-960 and Monsanto 30249 as well as with new materials and combinations and different methods of application

EVERGLADES EXPERIMENT STATION, PLANTATION FIELD LABORATORY,
FORT LAUDERDALE, FLORIDA

PHYSIOLOGIC RACES OF CROWN RUST OF OATS IDENTIFIED IN 1960[1]

M. D. Simons and L. J. Michel[2]

DESCRIPTION OF NEW RACES

Since the compilation of previously unnumbered races of crown rust (Puccinia coronata Cda. var. avenae Fraser & Led.) was published in 1960 (3), five new races have been discovered by the authors. Race 321 is of special significance because it is the first race reported to attack both the Landhafer and the Saia varieties. Race 325 also is potentially important. On the basis of reactions of the 10 standard differential varieties, race 325 belongs to race group 264. Reactions of certain supplemental differentials, however, indicate that it is perhaps a link between race groups 264 and 290. Race 324 attacks both Saia and Victoria, but not Landhafer. Races 322 and 323 are of no special interest from the standpoint of breeding oat varieties for resistance to crown rust.

PREVALENCE AND DISTRIBUTION OF RACES IN 1960

In 1960, as in the preceding few years, crown rust in the United States was light, but was scattered widely over the humid oat-growing States. Consequently, collections were received from Uniform Oat Rust Nurseries, other breeding and disease nurseries, and commercial fields representing most of the principal oat-growing regions of the United States. The 675 isolates identified from this material were distributed among 28 races (Table 1).

Table 1. Numbers of isolates of races of P. coronata var. avenae obtained from oats grown in different regions of the United States in 1960.

Race	North-central	North-eastern	South-central	South-eastern	% of total	Race	North-central	North-eastern	South-central	South-eastern	% of total
202	52	1	2	13	10.1	276	1				.1
203	23	2	7	9	6.1	280	1	3			.6
205			2		.3	281		2			.3
209		2			.3	284	1	4			.7
211	1				.1	285	1				.1
212	5	5			1.5	290	76		25	12	16.7
213	26		5	10	6.1	293			4		.6
216	143	6	58	32	35.4	295	63		4	9	11.3
231		1			.1	298	1				.1
237				7	1.0	320	3	6			1.3
240				1	.1	321	2	1			.4
258	1				.1	322	4		1		.7
264	15	1	5	7	4.1	323	1				.1
274	1				.1	325	6	1			1.0

About one-third of the 1960 isolates were identified as race 216. This race was more than twice as prevalent as the next most common race. The characteristics of race 216 and other common races mentioned in this report were discussed in earlier papers (2, 3). All races of the Victoria-attacking race group 216 (mainly races 213 and 216) made up about 43% of the total isolates as compared with 58% in 1959 and 70% in 1958.

Races of race group 290 (mainly races 290 and 295), which all parasitize Landhafer, made up over one-fourth of the total number of isolates. This represents a small, and probably insignificant, increase of these races as compared with their prevalence in 1959. The majority of these isolates induced moderately resistant, rather than resistant or highly resistant, reactions in such key sources of resistance as Victoria, Trispernia, and Bondvic.

[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Iowa Agricultural and Home Economics Experiment Station. Journal Paper No. J-4181, Project No. 1176, Ames, Iowa. The authors wish to acknowledge their indebtedness to the many cooperators who supplied crown rust collections for identification.

[2]Pathologist and Research Technician, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

The old Bond-attacking races (mainly races 202 and 203) accounted for 20% of the total isolates, an appreciable increase over 1959. Race 264, of interest because it attacks all known sources of seedling resistance among the hexaploid oats, made up 4% of the total. This race showed very little fluctuation in prevalence for the past 3 years.

Twenty races were identified from 114 aecial isolates identified in 1960. The most common races among these isolates were those attacking Landhafer (race group 290). Next in prevalence were the Bond races, followed by the Victoria races. Such striking differences in racial composition of the uredial and aecial material were discussed in more detail in an earlier report (2). One of the new races identified in 1960, race 324, was isolated from aecial material.

REACTIONS OF SUPPLEMENTARY DIFFERENTIAL OAT VARIETIES

Most of the crown rust collections received in 1960 were used for testing the reactions of 10 supplementary differential varieties (1). As in the past, the diploid C. I. 3815 and the tetraploid C. I. 7233 were resistant or moderately resistant to all isolates. The diploids Glabrota and C. I. 4747 were susceptible to only a small percentage of the total isolates, but these included races of race group 290. C. I. 4747 also showed an indefinite or rather intermediate reaction to certain isolates.

A hexaploid line derived from Saia was resistant to all isolates of the Landhafer-attacking races to which Saia was resistant. It was susceptible, however, to a few isolates of certain common races that do not attack Saia, indicating that it does not have the full resistance of Saia (4). It was also susceptible, as would be expected, to the new races 321 and 324. The reactions of Ascencao and P. I. 174513 were similar to those reported for 1959 (3).

Two varieties, Garry and Putnam 61, were known to be resistant to the biotypes of race 290 that were prevalent when that race was discovered. They were susceptible, however, to the majority of the 1960 isolates of race group 290 and now have little value as sources of resistance to these races. A line designated as Purdue 5877, furnished by J. F. Schafer, was known to carry the crown rust resistance of the Gray Algerian variety. It was susceptible to isolates of race group 264, but showed good resistance to all isolates of race group 290, and was also resistant to the new races 321 and 325.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE AND IOWA AGRICULTURAL AND HOME ECONOMICS EXPERIMENT STATION, AMES, IOWA

EVALUATION OF DBCP FORMULATIONS AND APPLICATION DEPTHS FOR ROOT-KNOT NEMATODE CONTROL AND PHYTOTOXICITY TO TOMATOES[1]

J. M. Good[2]

Summary

Phytotoxicity to tomatoes from DBCP applied at planting was related to depth of application. Phytotoxicity was less when DBCP was applied 12 inches below the soil surface than for a comparable application 5 inches deep. There was no significant difference in phytotoxicity between an emulsifiable concentrate and a granular Attaclay formulation of DBCP. Both depths of application and formulations gave excellent, equivalent control of Meloidogyne incognita acrita Chitwood, 1949.

The data reported herein are from the latest of a series of experiments begun in 1956 to evaluate methods of application, dosages, and time of application of 1,2-dibromo-3-chloropropane (DBCP) for control of several parasitic nemotode genera on selected field and truck crops in southern Georgia. This experiment was initiated to determine the effect of DBCP placement depth and type of formulation on phytotoxicity to tomatoes and control of M. incognita acrita Chitwood, 1949.

Several workers have shown that DBCP injury can be reduced by using minimum effective dosages (2, 3, 4) or by preplanting application instead of at-planting treatment (1, 2). There is also some evidence that the type of formulation used may influence phytotoxicity. Aycock and Sasser (1) recently reported that an emulsifiable concentrate of DBCP caused phytotoxicity to tomatoes, whereas a granular formulation did not. They also found that injury from DBCP was reduced when the chemical was placed in the soil several inches to the side of the planting row rather than directly beneath the row. Good (2) reported that phytotoxicity was not evident in 1958 and 1959 on a number of truck crops treated with DBCP at planting, but in 1960 severe phytotoxicity on the same crops occurred following at-planting treatment with DBCP. Phytotoxicity was attributed to unfavorable soil conditions, depth of application, or type of formulation used.

PROCEDURES

The soil of the test field was a Tifton sandy loam with a field capacity of 8.4 to 8.6% soil moisture (oven dry). The land was plowed several weeks before chemical application. The plots were fertilized by furrow placement on March 28, 1961 at which time standard control plots were treated with 1,3-dichloropropene and 1,2-dichloropropane (D-D mixture) by in-the-row treatment. DBCP[3] was applied at the rate of 15.9 ml of technical material per 100 feet along the row (equivalent to 0.5 gallon of technical material per acre for a row spacing of 44 inches). Formulations and depths of application varied as shown in Table 1. The liquid formulation was 50% by volume technical material as an emulsifiable concentrate. This was diluted with water and applied with a gravity-flow applicator. The granular formulation was 17.3% by weight technical material on Attaclay and was applied with a granular insecticide applicator. Each formulation was applied at two depths: 1) a deep application 12 inches below the soil surface and 9 inches below the tomato transplant roots, and 2) a shallow application 5 inches below the soil surface and 2 inches beneath the plant roots. Tomato transplants (Rutgers variety) grown in methyl bromide-treated seedbeds were set immediately after application of DBCP on April 19, 1961. At this time the soil moisture was 6.9% (oven dry), and the soil temperature at noon was 71° F at a 6-inch depth.

The experimental design was a randomized block with five replications of each soil treatment. Each plot was a single row 50 feet long. All data were analysed for statistical significance.

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[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Georgia Agricultural Experiment Stations.

[2]Senior Nematologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

[3]Furnished by the Shell Oil Company, Agricultural Chemical Division.

Table 1. Effect of DBCP application depth and type of formulation on plant vigor rating, root-knot indices, and tomato yields.

Soil treatment	Average plant vigor ratings[a] May 2, 1961	Average root-knot indices[b] July 25, 1961	Average tomato yield (pounds/plot)
Emulsifiable DBCP			
shallow (5 inches)	2.00	0.05	29.3[c]
deep (12 inches)	2.12	0.00	35.9
Granular DBCP			
shallow (5 inches)	1.00	0.03	26.5
deep (12 inches)	2.10	0.04	20.8
D-D mixture control	3.50	0.06	34.8
Untreated check	4.00	3.10	65.0
LSD .05	0.85	0.75	17.5
LSD .01	1.17	1.02	23.9

[a]Vigor ratings of 1-7 (1, severe stunting; 2, moderate stunting; 3, slight stunting; 4, controls or normal growth; 5, slightly improved; 6, good growth; 7, excellent growth).
[b]Root-knot index of 0-4 (0, clean; 1, trace; 2, light; 3, moderate; 4, heavy galling).
[c]Average yield per plot for total of six pickings.

Visual ratings of plant vigor were made for all treatments 2 weeks after planting. Within each replication, the chemically treated plots were compared with an untreated check plot, which was arbitrarily given a rating of 4. Plots that were inferior to the checks or showed phytotoxicity were rated 3 (slight stunting), 2 (moderate stunting), or 1 (severe stunting). Plots that showed improved vegetative growth were rated 5 (slightly improved), 6 (good), and 7 (excellent).

Yields were recorded as pounds of fruit harvested in six pickings from each plot.

After the final picking all plants were dug and rated for severity of root-knot nematode infection by a galling index system of 0 to 4 (0, clean; 1, trace; 2, light; 3, moderate, and 4, heavy galling).

The average values for plant vigor, yield of fruit and root-knot index are shown in Table 1.

RESULTS AND DISCUSSION

Root-knot control was excellent for all chemical treatments, with no significant difference between D-D and DBCP treatments, for depths of application or formulation of DBCP. The best control (not significant) was obtained with emulsifiable DBCP applied at a 12-inch depth.

Plant vigor ratings made in May, 2 weeks after transplanting, indicated that all DBCP treatments caused significant injury to tomatoes. Phytotoxicity tended to be greatest for shallow placement for both formulations, but difference was significant only for the shallow application of granular DBCP. There was no significant difference in phytotoxicity for the two formulations of DBCP; however, the plant vigor ratings and field observations indicated that the granular formulation tended to give somewhat more phytotoxicity than did the emulsifiable concentrate (Table 1, Figs. 1-3).

Phytotoxicity from DBCP was evident throughout the growing season, but differences between the formulations and depths of application largely disappeared by the end of the season. All DBCP treatments significantly reduced yields, but there was only a small, non-significant difference in yield between the various DBCP treatments. Yield of tomatoes from D-D treated plots was also significantly lowered. This was attributed to poor nitrification, rather than to chemical injury to the plants.

The data reported here indicate that shallow placement, as is customary with fertilizer-nematocide mixtures, can increase the chances of DBCP injury, particularly to moderately sensitive crops during wet, cool weather. Deeper placement of DBCP should lessen the possibility of phytotoxicity to a number of crop plants.

FIGURE 1. A -- Row treatment with emulsifiable DBCP applied 12 inches below soil surface. B -- Row treatment with emulsifiable DBCP applied 5 inches below soil surface.

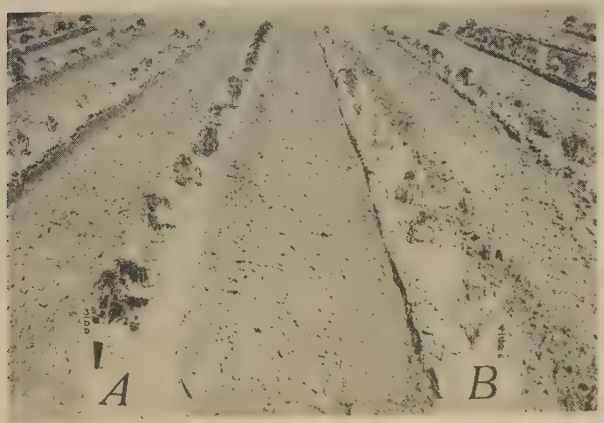

FIGURE 2. A -- Standard preplanting row treatment with D-D. B -- DBCP impregnated clay granules applied 5 inches below the soil surface.

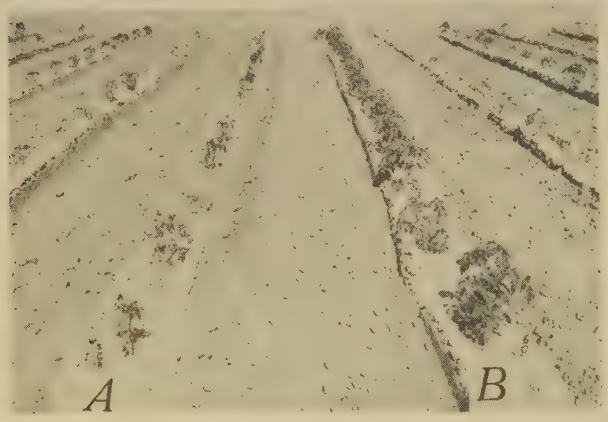

FIGURE 3. A -- DBCP impregnated clay granules applied 12 inches below the soil surface. B -- Untreated control.

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UNITED STATES DEPARTMENT OF AGRICULTURE, NEMATOLOGY INVESTIGATIONS, GEORGIA COASTAL PLAIN EXPERIMENT STATION, TIFTON, GEORGIA

A STEMPHYLIUM LEAF SPOT DISEASE OF GRAM

G. N. Das and P. K. Sen Gupta

A leaf spot disease of gram (Cicer arietinum) caused by a species of Stemphylium has been observed at the State Agricultural Farm, Berhampore, West Bengal during February 1961. As there is no report of the fungus on gram, a study of the pathogen was undertaken.

Lesions on the leaflets in the field consist of somewhat ovoid necrotic spots which may measure up to 6 x 3 mm. The spots are dark brown at the center, surrounded by a larger ashy border. Spots also develop on stems and these appear as minute dark brown elongated areas.

Conidiophores arise singly, they are dark brown in color, are septate, and have swollen tips. The size of the conidiophores varies from 37-70 x 5-7μ with 2 to 5 septa. Conidia are borne singly at the tips of the conidiophores; they are subglobose to ovoid, olive brown in color, smooth walled, constricted at the median septum and with transverse, longitudinal and oblique septa; tips of the conidia are rounded. The conidia fall within the range of 20-30 x 17-27μ (average 23.3 x 19.9μ).

CULTURAL STUDIES

The fungus was found to grow very slowly. Of the various media tested, namely potato-dextrose, oatmeal, gram, Richard's and Brown's agar, best growth was obtained in potato-dextrose agar. In this medium, also, growth was very slow; radial growth was only 20 mm in 10 days.

The optimum temperature for mycelial growth was found to be 25° C.

On PDA the colonies were greyish in color with a somewhat irregular margin, more or less appressed with rather sparse aerial mycelium which is dull white in color and velvety in texture.

INOCULATION STUDIES

Seedlings of gram, red clover (Trifolium pratense), white clover (T. repens), sweet clover (Melilotus alba), alfalfa (Medicago sativa), and blue lupine (Lupinus angustifolius) were inoculated with conidial and mycelial suspensions of the fungus. For each test seedlings in three pots were inoculated with the fungus and covered with bell jars for 48 hours.

Spots on gram leaves appeared 4 days after inoculation. On other plants no symptom was produced.

IDENTIFICATION OF THE FUNGUS

The morphological description of the fungus conforms to that of Stemphylium sarcinaeforme (Cav.) Wiltsh. The reported hosts of Stemphylium sarcinaeforme are red clover (1, 4) and blue lupine (5). White clover, however, was reported to be successfully inoculated with S. sarcinaeforme (2, 3). In the inoculation tests conducted in this laboratory none of these reported hosts of S. sarcinaeforme was infected by the fungus isolated from gram. Since the fungus isolated from gram is similar morphologically to Stemphylium sarcinaeforme, but failed to infect red clover and lupine, the reported hosts of the fungus, the pathogen isolated in this laboratory appears to be a new forma of Stemphylium sarcinaeforme.

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STATE AGRICULTURAL RESEARCH INSTITUTE, WEST BENGAL, CALCUTTA, INDIA

FIRST REPORT OF PINE MORTALITY CAUSED BY FOMES ANNOSUS ROOT ROT IN OHIO

Thomas W. Jones[1]

Root rot caused by Fomes annosus Fries has killed some trees in a mixed plantation of shortleaf pine (Pinus echinata) and Virginia pine (P. virginiana) in Hocking County, Ohio. This is believed to be the first report of Fomes root rot in Ohio.

The affected plantation, 43 acres in extent, was established in 1939. About 10 acres were lightly thinned for pulp in January to March of 1956. The first mortality occurred about 3 years later. All dead and dying trees are in one small group less than 0.1 acre in size in the thinned portion of the stand. Both shortleaf and Virginia pines are affected. Fruiting bodies of the causal fungus were found at the base of several of the recently killed trees.

CENTRAL STATES FOREST EXPERIMENT STATION, FOREST SERVICE,
UNITED STATES DEPARTMENT OF AGRICULTURE

[1]Plant Pathologist, United States Department of Agriculture, Forest Service, Central States Forest Experiment Station, Forest Disease Laboratory, Delaware, Ohio.

OUTBREAK OF CURLY TOP IN COSTA RICA

Ricardo A. Rodriguez[1]

What is believed to be the curly-top virus disease was shown to occur on tomatoes in the plantations of Paraiso, S.W. of Cartago, Costa Rica. There is no previous report of the disease in this country, and no information in this respect has been obtained from other Central American countries.

The disease first appeared this year at the start of the rainy season in the middle of July, and affected the crop severely. Plants became very unproductive and fruits were malformed. The symptoms of leafrolling along the midrib and purple veins at the margin of the leaflets were typical of the disease. Other characteristics of the disease were the same as those described by Doolittle[2] for tomato.

The disease was readily transmitted to healthy plants by an approach graft; the whole syndrome became evident approximately 5 weeks later. However, tests with the insect vector are necessary to determine whether this is the North or South American strain of the virus Ruga verrucosans Carsner & Bennet.

SECCION DE FITOPATOLOGIA, MINISTERIO DE AGRICULTURA Y GANADERIA,
SAN JOSÉ, COSTA RICA

[1]Plant Pathologist and Head, Seccion de Fitopatologia, Ministerio de Agricultura y Ganaderia, San José, Costa Rica.

[2]Doolittle, S. P. 1948. Tomato Diseases. United States Department of Agriculture. Farmer's Bull. No. 1934.

ROOT-KNOT NEMATODE ON DIOSCOREA IN GUATEMALA[1]

Eugenio Schieber and Dorothea Lassmann K.

FIGURE 1. Aerial symptoms produced by M. incognita on Dioscorea composita.

Since the first report[2] of parasitic nematodes found on dioscorea plantations in the Pacific Coast of Guatemala, further surveys and observations have been made.

Soil samples and tubers collected at Finca "Honduras" and Finca "Las Delicias" were processed in the laboratory. Meloidogyne incognita (Kofoid & White) Chitwood was constantly found as the main species attacking Dioscorea floribunda, D. composita, and D. spiculiflora. Other species reported previously are not the predominant ones. Inoculation work with M. incognita was carried out in the greenhouse. Symptoms were severe on tubers as well as on aerial parts of the plant (Fig. 1).

Further surveys on the Pacific Coast revealed that M. incognita constitutes a potential problem for the establishment of this crop in Guatemala.

INSTITUTO AGROPECUARIO NACIONAL, GUATEMALA C. A.

[1]Acknowledgment is given to Dr. Gerald Thorne of the University of Wisconsin for confirming the identification.

[2]Eugenio Schieber. 1961. Parasitic nematodes on dioscorea in Guatemala. Plant Disease Reptr. 45: 425.